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Using poststratification to improve abundance estimates from multispecies surveys: a study of juvenile flatfishes

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Abstract—Population assessments seldom incorporate habitat information or use previously observed distributions of fish density. Because habitat affects the spatial distribution of fish density and overall abundance, the use of habitat information and previous estimates of fish density can produce more precise and less biased population estimates. In this study, we describe how poststratification can be applied as an unbiased estimator to data sets that were collected under a probability sampling design, typical of many multispecies trawl surveys. With data from a multispecies survey of juvenile flatfish, we show how poststratification can be applied to a data set that was not collected under a probability sampling design, where both the precision and the bias are unknown. For each of four species, three estimates of total abundance were compared: 1) unstratified; 2) poststratified by habitat; and 3) poststratified by habitat and fish density (high fish density and low fish density) in nearby years. Poststratification by habitat gave more precise and (or) less design-biased estimates than an unstratified estimator for all species in all years. Poststratification by habitat and fish density produced the most precise and representative estimates when the sample size in the high fish-density and low fish-density strata were sufficient (in this study, $n \geq 20$ in the high fish-density stratum, $n \geq 9$ in the low fish-density stratum). Because of the complexities of statistically testing the annual stratified data, we compared three indices of abundance for determining statistically significant changes in annual abundance. Each of the indices closely approximated the annual differences of the poststratified estimates. Selection of the most appropriate index was dependent upon the species' density distribution within habitat and the sample size in the different habitat areas. The methods used in this study are particularly useful for estimating individual species abundance from multispecies surveys and for retrospective studies.

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Scientists must be able to assess population abundance with a high degree of confidence to achieve the goals of fishery management (Quinn, 1985). To do this, survey designs and estimation methods that minimize the variance in estimates of abundance are needed. Recently, the National Research Council (NRC, 2000) recommended incorporating habitat information and commercial fisheries data in population assessments. Both of these data may result in lower variances in estimates of abundance.

Habitat type and habitat quality are becoming more widely recognized as primary determinants for the distribution and survival of marine fish species (Murawski and Finn, 1988; Gadomski and Caddell, 1991; Reichert and van der Veer, 1991; Norcross et al., 1999). Until recently, however, few studies have been directed toward defining fish habitat or using habitat associations to help decrease the variability in abundance estimation (Scott, 1995). In response to the growing recognition of the importance of habitat, the Magnuson-Stevens Fishery Conservation and Management Act was amended in 1996 (Public Law 104–297) so that the National Marine Fisheries Service (NMFS) and regional fishery management councils must describe and identify essential fish habitat (EFH) for managed species. Similarly, a recent report from the NRC calls for methods that link environmental data to stock assessments (NRC, 2000).

Poststratification can be used in a number of different ways to address the NRC recommendations. Although poststratification is not a new statistical method, it is one that is not commonly used for estimating groundfish population abundance and can be used to meet these newly defined challenges. In contrast to a stratified sampling design, poststratification is a method that allocates samples to strata after they have been collected. As a result, habitat data collected during a survey can be used for stratification. When poststratification is applied to data that have been collected under a simple random sampling design, the poststratification estimator is unbiased and may produce more precise estimates than those from a simple random sampling estimator. Poststratified estimates will be nearly as precise as stratified sampling with proportional allocation, in which the sample sizes in each stratum are proportional to stratum sizes, if stratum sample sizes are large ($n > 20$) and errors in estimates of strata areas are negligible (Cochran, 1977; Pollock et al., 1994;

Scheaffer et al., 1996). If poststratification is applied to data from a multispecies survey, 1) abundance data for each species can be poststratified with different habitat variables or 2) abundance data for every species can be poststratified with the same variables, but different stratum boundaries can be used for each species.

Many large-scale multispecies groundfish surveys are conducted by using a stratified random sampling design (Azarovitz, 1981; Halliday and Koeller, 1981; Pitt et al., 1981; Martin[1]; Weinberg et al.[2]). Depth, distance from or along shore, latitude, distance along depth contours, or broad geographic features (such as bays, capes, banks, gullies, and slopes) are used as stratum boundaries in trawl surveys because they have been shown to be related to species distributions. These factors are fixed spatially, allowing samples to be allocated to strata prior to sampling. The same boundaries are used for all species, and boundaries generally remain the same over years.

When conducting a multispecies survey with a stratified random sampling design, optimal stratification for one species may not be optimal for others (Koeller, 1981; NRC, 2000). Because the placement of strata boundaries is critical for precise stratified estimates (Cochran, 1977), use of a stratified sampling design for a multispecies survey may result in only small gains in precision for some or all species. Poststratification is possible for data that have been collected under a stratified design. It can be used to stratify data more finely for individual species. Under stratified random sampling, a simple random sample is taken in each stratum. Thus, data within each stratum can be poststratified separately with additional variables and the abundance estimates from each of the strata can be summed. The resultant estimator is unbiased and likely will be more precise than that of the original stratified design if sample sizes in poststratified strata are large enough.

Often, researchers need to estimate abundance from data sets that were not recorded under a probability sampling design (a design in which randomness is built into the survey design, such as simple random sampling or stratified random sampling). Finances and logistics, for example, may make it impossible to collect data under a probability sampling design, researchers may want to estimate species abundance from commercial fisheries or other nonsurvey data, or previously collected data sets that were not recorded under a probability sampling design may be used for retrospective studies. In this article, we refer to data collection without a probability sampling design as "haphazard sampling." The use of haphazardly collected data for estimating abundance is undesirable because they cannot be evaluated by the theorems of probability theory (Krebs, 1989). Although undesirable, it is often necessary to analyze haphazardly collected data and effective methods are needed to do so.

Poststratification can be applied to data that were not collected with a probability sampling design. When poststratification is applied to data not collected under a probability sampling design, the poststratification estimator, a design-based estimator, may be biased. When analyzing such data, it is important both to maximize the precision and to minimize the bias. Poststratification has been applied to nonprobability samples in other studies to increase the precision (Hall and Boyer, 1988) and decrease the bias of estimators (Buckland and Anganuzzi, 1988; Hall and Boyer, 1988; Anganuzzi and Buckland, 1989).

Poststratification can be useful, but has some drawbacks. With poststratification, sample sizes within strata are random variables—which are an additional source of variability over that of a stratified sampling variance estimator (Thompson, 1992; Scheaffer et al., 1996). The variance of a poststratified estimator can be estimated by using standard stratified sampling variance equations and by incorporating an additional approximate term to account for the random sample sizes present with poststratification (Scheaffer et al., 1996). Alternatively, the variance of a poststratified estimator can be estimated by conditioning on samples sizes and by applying the standard stratified sampling variance equation (Thompson, 1992). For accurate poststratification estimates, the proportion of total possible samples in each stratum (for this study the proportion of the total survey area included in each stratum) must be known or approximated closely enough that the error in the approximation is negligible (Cochran, 1977). Error in estimates of stratum sizes causes bias in poststratified estimates of abundance. Because error in the estimation of stratum size is unaccounted for in the estimated variance of poststratified estimates, the estimated variances may be underestimates of the true error (Cochran, 1977).

This study had two goals. The first goal was to evaluate the benefits and drawbacks of using poststratification to incorporate habitat and fish-density information into estimates of abundance from multispecies survey data that were not collected under a probability sampling design. To achieve this goal, this study compared three estimates of total abundance and variance (unstratified, poststratified by habitat, poststratified by habitat and estimates of fish density in neighboring years) for each of four species. The comparison was made to determine whether poststratification of haphazardly sampled data with habitat and fish-density information increases the precision and helps account for possible bias in abundance estimates.

[1] Martin, M. H. 1997. Data report: 1996 Gulf of Alaska bottom trawl survey. NOAA Tech. Memo. NMFS-AFSC-82, 235 p. National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, Virginia 22161.

[2] Weinberg, K. L., M. E. Wilkins, R. R. Lauth, and P. A. Raymore jr. 1994. The 1989 Pacific west coast bottom trawl survey of groundfish resources: Estimates of distribution, abundance, and length and age composition. NOAA Tech. Memo. NMFS-AFSC-33, 168 p., plus appendices. National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, Virginia 22161.

Because this study is an observational study with haphazard sampling, the precision and bias cannot be directly assessed. Instead, we estimated and compared the precision by using unstratified and poststratified estimators. We qualitatively estimated the relative amount of design bias (i.e., how representative the estimates are) with the use of habitat. In previous studies (Norcross et al., 1995; 1997; 1999), depth and sediment were identified as habitat characteristics closely associated with the distribution of the four species in this study. From depth, sediment, and fish abundance data collected in this study we were able to identify ranges of habitat characteristics associated with areas of high, low, and no fish density. By estimating the proportion of area (km^2) in the study area characterized by the ranges of depth and sediment, it was possible to estimate the proportion of the survey area with high, low, and no fish density. Because samples in our study were not randomly allocated, the probability of selection was not equal among all samples in the survey area. The resulting numbers of samples taken in areas of high, low, and no fish density were not in proportion to the size (km^2) of those areas as it would have been with repeated simple random sampling. Therefore, by comparing the relative size of high, low and no fish-density areas in the survey area with the relative number of samples in those areas, we made qualitative estimates of the design bias associated with the estimators. Although an assessment of the relative amount of design bias made in this way is only an approximation, it is helpful when using haphazardly collected data in order to provide some indication of the amount of design bias based on the disproportion of samples in an area to the size of that area.

Figure 1

Study area (in black) in Middle and Kalsin Bays, Kodiak Island, Alaska.

Because of the complexities of statistically testing the annual stratified data, the second goal of our study was to develop indices of abundance that closely approximated the annual differences of poststratified estimates and that could easily be tested for statistically significant changes between years. To achieve the second objective, three indices of annual relative abundance were constructed and compared with respect to their estimated relative precision and design bias: one from all sites in the survey area, one from all sites within the species' habitat, and one from all sites within an area of high fish density within the species' habitat.

The data for this study were obtained from six years of juvenile groundfish surveys conducted in Kalsin Bay and Middle Bay, Kodiak Island, Alaska. The four species studied were age-0 rock sole (*Lepidopsetta* spp.), age-1 yellowfin sole (*Pleuronectes asper*), age-0 Pacific halibut (*Hippoglossus stenolepis*), and age-0 flathead sole (*Hippoglossoides elassodon*). The survey data were collected during the six-year survey under three different survey designs, none of which were strictly randomized, but each involved some degree of haphazard sampling due to weather, sediment structure, and other logistical restrictions for beam trawling in small bays off the Gulf of Alaska (Norcross et al.[3]). Although many trawl survey data sets to which these methods could be applied are collected under a probability sampling design where the estimator is unbiased, the haphazardly collected data set used in our study was chosen to show how poststratification can be applied when both the precision and the bias of the estimator are unknown.

Methods

Sampling

Middle and Kalsin Bays are part of Chiniak Bay, 10 nmi south of the town of Kodiak, Alaska. The total size of the study area, 87 km^2, included the combined areas of both bays and the areas directly outside the mouths of the bays (Fig. 1). Middle Bay is 8 km long and has depths of 50 m at the mouth of the bay and an area of 21 km^2. Kalsin Bay is 8 km long, has depths greater than 100 m

[3] Norcross, B. L., B. A. Holladay, A. A. Abookire, and S. C. Dressel. 1998. Defining habitats for juvenile groundfishes in Southcentral Alaska with emphasis on flatfishes. Vol. I, Final Study Report, OCS Study MMS 97-0046, 131 p. Coastal Marine Institute, Univ. Alaska Fairbanks, Fairbanks, AK 99775.

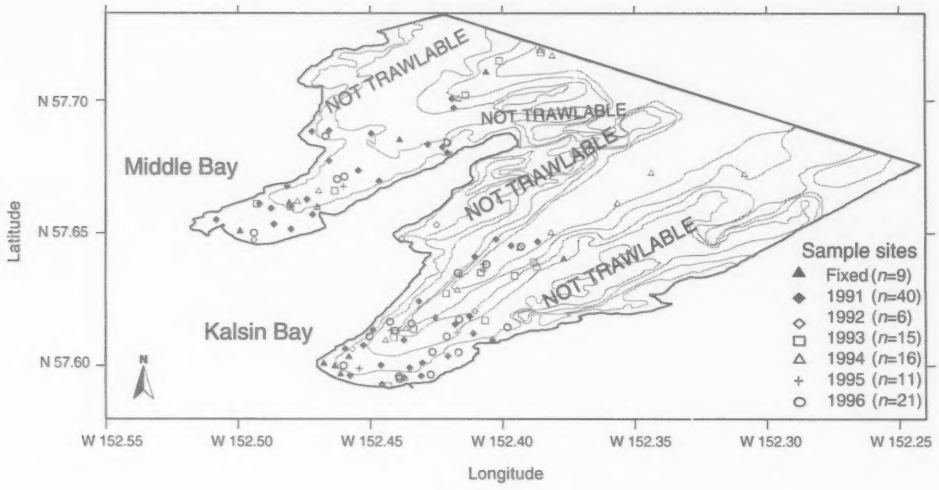

Figure 2

Kalsin and Middle Bay sample sites (1991–96) and bathymetry. Fixed (sampled every year) sites are noted.

at the mouth of the bay, and encompasses an area of 34 km^2. Rocky cliffs and islands surround the mouths of the bays, and rocks in the sediment made several areas untrawlable (Fig. 2). Although trawling was not conducted in these areas, depth and sediment data were collected. In this analysis, untrawlable areas were still considered possible flatfish habitat and were included in the measurements of the size of the total study area.

Annual cruises were conducted in Middle and Kalsin Bays for two weeks in August from 1991 to 1996. Juvenile flatfish were collected by using 3.05 and 3.66 m plumb-staff beam trawls (Gunderson and Ellis, 1986). Trawl nets were made of 7-mm square net mesh and had a 4-mm codend liner that retained flatfish as small as 11 mm. Sampling methods were consistent for all six years (Norcross et al., 1995; Norcross et al.[3]). Collections at each sample site included a tow of 10 minutes or less, a vertical CTD (conductivity, temperature and depth) cast, and a sediment grab (0.06-m^3 Ponar grab). The sampling area of each tow was determined by the width of the beam trawl, which was 0.74 of the beam length (Gunderson and Ellis, 1986), and distance towed was based on global positioning system (GPS) coordinates. Fish were identified to the lowest possible taxon and measured to the nearest millimeter total length. At the time of collections, all rock sole were identified as *Pleuronectes bilineatus*. Following Orr and Matarese's (2000) revision of the genus, we refer to these fishes as *Lepidopsetta* spp. in this article because both species, *L. bilineata* and *L. polyxystra*, were identified in the study area during 1996 sampling. Fish ages were determined by length-frequency analysis. Fish catch-per-unit-of-effort (CPUE) values were standardized to a 1000-m^2 tow area.

Sampling designs varied from year to year (Norcross et al.[3]). Extensive exploratory sampling was conducted from 1991 through 1994 to describe juvenile flatfish distributions in relation to habitat characteristics (Norcross et al., 1995; 1997). The goal in these years was to sample over the widest range of areas and habitat characteristics possible within the depth, sediment, weather, and logistical constraints. In 1995 and 1996, sampling was stratified by depth and percent sand in sediment. The sample allocation and the number of strata differed in 1995 and 1996 (Norcross et al.[3]). Because of logistical constraints, samples were not randomly allocated within each stratum. Within these sampling designs, nine fixed sites were chosen, each with different depth and sediment combinations and with high abundances of one of the four species. Each of the nine fixed sites was sampled at least once in each of the six years. For this study, survey data in each year were treated as unstratified samples that were not collected under a probability sampling design.

Analysis

Poststratification Habitat preferences of juvenile flatfishes, as defined by depth and sediment variables, have been identified as affecting the distribution and abundance of juvenile flatfish around Kodiak Island (Norcross et al., 1995; 1997; 1999; Mueter and Norcross, 1999) and elsewhere (Pearcy, 1978; Tanda, 1990; Burke et al., 1991; Rogers, 1992; Walsh, 1992). Four areas were defined for use in estimating total and relative abundance: habitat, nonhabitat, high fish-density (HFD) and low fish-density (LFD) areas. Percent sand was used as a continuous variable of sediment type. Suitable habitat (habitat area) was defined for each species as ranges of depth and percent sand in which the species was caught during one or more

of the six sampling years. Unsuitable habitat (nonhabitat area) was defined for each species as ranges of depth and percent sand in which the species was never caught. Within the habitat area, the area of high fish density for each year was defined as ranges of depth and percent sand associated with CPUEs in the 75th–100th percentile of nonzero catches in the five other years. The area of low fish density was defined as the remaining habitat area not incorporated in the HFD area.

In order for the poststratification method to estimate abundance accurately (high precision and low bias), the size of each stratum must be known or closely approximated (Cochran, 1977; Scheaffer et al., 1996). When using habitat variables to determine stratum sizes, the accuracy of stratum sizes defined by the boundaries is heavily dependent upon the number and distribution of habitat variable measurements. For our study, 243 depth and percent sand measurements collected over the six years at trawl locations were used to determine stratum boundaries. The ranges of depth and percent sand that defined the four areas for each species were contoured over the study area by using a minimum curvature algorithm (Surfer, 1995). The size of each stratum in relation to the size of the entire study area was then visually estimated to the nearest square kilometer. Although not used in our study, a digital representation of the size of each stratum and the size of the study area is recommended to produce more precise estimates.

To assess the advantages and disadvantages of using poststratification to estimate abundance, three estimates of total abundance were calculated and compared for each species in each year. An unstratified estimate of total abundance was calculated from samples across the entire survey area, with no differentiation with regard to habitat. The unstratified estimate of total abundance was calculated with the standard simple random sampling equation

$$\hat{\tau} = N\bar{y},$$

where $\hat{\tau}$ = the estimated population total;
N = the total number of possible samples in the survey area; and
$\bar{y}$ = the mean CPUE of all sites sampled in a year.

The estimated variance for the unstratified estimator was calculated as

$$\hat{V}(\hat{\tau}) = N^2\left(\frac{s^2}{n}\right)\left(\frac{N-n}{N}\right),$$

where $\hat{V}(\hat{\tau})$ = the estimated variance of the population total estimate;
N = the total number of possible samples in the survey area;
n = the total number of samples taken; and
s^2 = the sample variance.

The estimate poststratified by habitat was calculated as

$$\hat{\tau}_{st} = \sum_{i=1}^{L} N_i \bar{y}_i,$$

where $\hat{\tau}_{st}$ = the estimated population total;
L = the number of strata (here L=2, habitat and nonhabitat);
N_i = the total number of possible samples in stratum i (samples were standardized to 1000 m^2, therefore $N_i \times 1000$ m^2=stratum size); and
$\bar{y}_i$ = the mean CPUE in stratum i.

A third estimate, poststratified by habitat and fish density, was calculated with the same poststratification estimator with L=3. This poststratification estimator used the HFD area of that year as one stratum, the LFD area of that year as the second stratum, and the nonhabitat area as the third. An approximate variance estimator (Scheaffer et al., 1996),

$$\hat{V}_p(\hat{\tau}_{st}) = \frac{N(N-n)}{n}\sum_{i=1}^{n}\frac{N_i}{N}s_i^2 + \frac{N^2}{n^2}\sum_{i=1}^{n}\left(1-\frac{N_i}{N}\right)s_i^2,$$

was used to estimate the variance of each poststratification estimator,

where $\hat{V}_p$ = the estimated poststratified variance of $\hat{\tau}_{st}$, the estimated population total;
N = the total number of possible samples in the survey area;
n = the total number of samples taken;
N_i = the total number of possible samples in stratum i; and
s_i^2 = the sample variance in stratum i.

The first term of the variance equation is the variance of a stratified sample mean under proportional allocation. The second term shows the amount of increase in variance expected from post- rather than prestratification (Scheaffer et al., 1996).

Relative efficiency statistics were calculated for pairwise comparisons of the precision of the unstratified and the two poststratified estimates. Pairwise comparisons of the estimates were made for each species in each year. Relative efficiency was calculated as

$$R.E. = \frac{V_A}{V_B},$$

where V_A represents the variance of an unstratified estimate or a stratified sample with fewer strata than the estimate of variance represented by V_B.

The variance of an estimate is directly affected by the sample size (Zar, 1996). In our study, three total abundance estimates and their respective variances were

calculated and compared for each of the 24 species-year combinations. One of the three total abundance estimates was most precise for each of the species-year combinations. For each species-year combination, the habitat stratum sample size (used in the estimate post-stratified by habitat), the HFD stratum sample size, and the LFD stratum sample size (both used in the estimate poststratified by habitat and fish density) were plotted in relation to the total abundance estimator that was most precise in order to investigate the influence of sample size on the relative precision of the three total abundance estimators.

Indices of abundance Three indices were constructed for each species in each year to determine interannual variations in relative abundance (mean CPUE): an all-site index, a habitat index, and a HFD index. For each species and year, the all-site index was the mean CPUE from all sites sampled. The habitat index was the mean CPUE from all sites sampled within the species' habitat area. The HFD index was the mean CPUE from all sites sampled within the species' HFD area.

CPUE values were not normally distributed and therefore the Kruskal-Wallis nonparametric analysis of variance test was used to test the three indices for each species' differences in mean CPUE among years. For species that showed significant differences (α=0.05), a Tukey HSD (honestly significant difference) multiple comparison test for unequal sample sizes was conducted to determine which years differed (α=0.05). The Tukey multiple comparison test was used because it is robust with respect to departures from population normality and homogeneity of variance (Keselman, 1976). The results for the three indices for each species were compared to see how the differences in estimating abundance with the three indices affected conclusions of significant differences in abundance between years.

Numerous sources of bias can affect estimators of abundance from survey data. The poststratification estimator and other design-based estimators may be biased when applied to data that were not collected under a probability sampling design, as done in the present study. For a qualitative estimate of possible design bias in the estimates, the annual proportion of sample sites in each stratum (habitat, nonhabitat, HFD, and LFD strata) were compared with the proportion of area (km^2) in that stratum. First, we compared the size of the habitat area, in relation to the size of the total survey area, with the number of samples taken in the habitat area, in relation to the number taken in the total survey area.

$$\frac{\text{Size of the habitat area}}{\text{Size of the total survey area}} : \frac{\text{Number of samples taken in the habitat area}}{\text{Number of samples taken in the total survey area}}$$

Second, we compared the size of the HFD area, in relation to the size of the total habitat areas, with the number of samples taken in the HFD area, in relation to the number taken in the total habitat area.

$$\frac{\text{Size of the HFD area}}{\text{Size of the habitat area}} : \frac{\text{Number of samples taken in the HFD area}}{\text{Number of samples taken in the habitat area}}$$

Recognizing that the distribution of individuals varied within and across strata, two measures were used to better understand the distribution of each species in each year. The proportion of zero catches (e.g., a "zero catch" for rock sole indicates a tow in which no rock sole were caught) and the mean CPUE of nonzero catches were calculated for each species in each year over four areas: the total survey area, the habitat area, the HFD area, and the LFD area.

Results

Fish CPUE statistics were calculated for a total of 244 quantitative tows over the six sampling years (Fig. 2) in habitats ranging from 1 to 111 m depth and from 0% to 99% sand. Based on compiled data from all six years, the habitat area for rock sole was defined by 1–84 m depth and 2–99% sand; for yellowfin sole, by 2–43 m depth and 24–99% sand; for Pacific halibut, by 2–27 m depth and 2–99% sand; and for flathead sole, by 12–87 m depth and 8–97% sand (Fig. 3). The HFD area, defined by depth and percent sand, was determined for each of the four species in each of the six years (Table 1, Fig. 3). Although the range of depth and the range of percent sand were determined independently in each year, they remained quite constant for each species over the six sampling years.

The size of habitat area in relation to total area ranged across species from 0.62 to 0.92 and, for each species, the proportion of habitat sites to total sites varied among years (Table 2). The proportion of sample sites in habitat to sample sites in the total survey area ranged from 0.88 to 1.00 for rock sole, 0.60 to 0.87 for yellowfin sole, 0.52 to 0.93 for Pacific halibut, and 0.29 to 0.67 for flathead sole. The relative number of samples taken in each species' habitat area exceeded the relative size of their habitat area (i.e., a positive disproportion of samples in habitat), except for rock sole in 1991 and 1994, yellowfin sole in 1993 and 1994, Pacific halibut in 1993 and 1994, and all years for flathead sole. On average, rock sole had a 5% positive disproportion of samples in its habitat area, yellowfin sole and Pacific halibut had an 11% positive disproportion of samples in their habitat area, and flathead sole had a 15% negative disproportion of samples in its habitat area.

The size of the HFD area in relation to habitat area, and the number of sites sampled in the HFD area in relation to the number sampled in the entire habitat area, varied over the six sampling years for each of the four species (Table 2). On average over the six years,

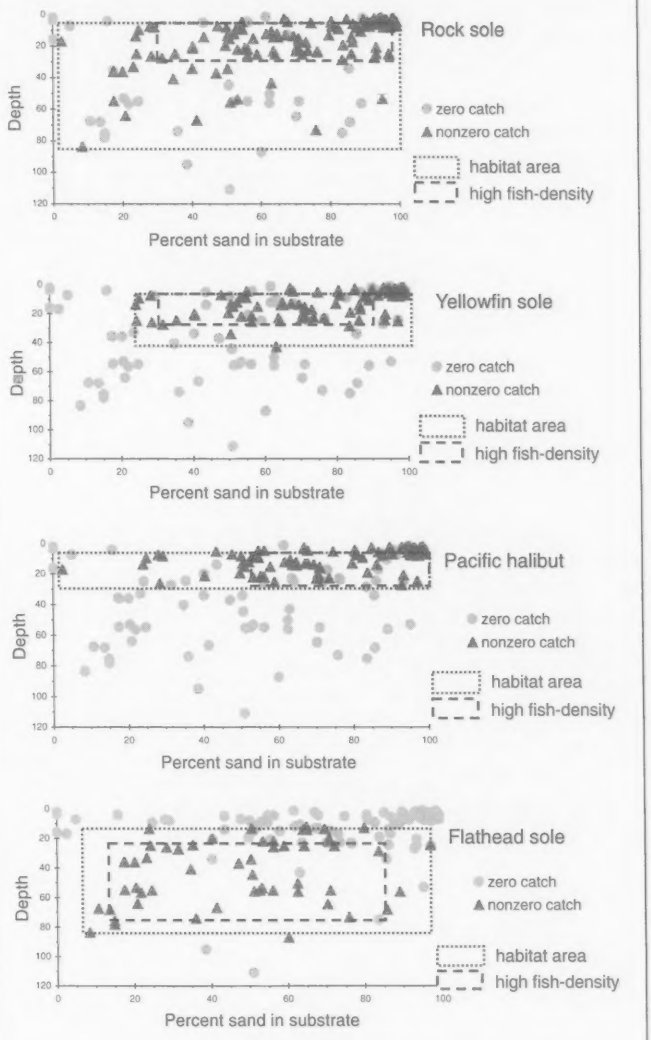

Figure 3

Summary of 1991–96 tows, in relation to depth and percent sand. Tows are divided into zero and nonzero catches for each species. The dotted line separates the depth and percent sand characteristics of habitat and nonhabitat areas. The dashed line separates the depth and percent sand characteristics of high and low fish-density areas within the habitat area.

rock sole had a 10% negative disproportion of samples in the HFD area, Pacific halibut had a 3% negative disproportion of samples in the HFD area, and flathead sole had a 28% negative disproportion of samples in the HFD area. For yellowfin sole, the average distribution of samples between the high and low fish-density areas was in direct proportion to the size of the areas, i.e., there was no disproportion of samples.

Two measures were used to characterize the distribution of a species within their habitat: the proportion of zero catches and the mean of nonzero catches in high and low fish-density areas. As expected, for all species the average proportion of zero catches over all sites was greater than the proportion of zero catches in the habitat or HFD areas (Table 3). For rock sole, yellowfin sole, and flathead sole, the average proportion of zero

Table 1

Characteristics defining 1991–96 high fish-density areas for each species of flatfish. Ranges of depth and percent sand, defining the high fish-density (HFD) area, and the associated spatial coverage within the bay (km^2). Each year's HFD area was determined as the range of depth and percent sand associated with the 75th–100th percentile of nonzero catch from the other five years.

		Depth (m)		Percent sand in sediment		
Species	Year	minimum	maximum	minimum	maximum	Size (km^2)
Rock sole	1991	3.0	27.3	31.5	99.2	52
(*Lepidopsetta* spp.)	1992	3.0	36.0	20.2	99.2	56
	1993	3.0	27.3	31.5	99.2	52
	1994	3.0	27.3	31.5	98.8	52
	1995	3.0	27.3	31.5	99.2	52
	1996	3.0	25.0	47.8	99.2	46
	average	3.0	28.3	32.4	99.2	52
Yellowfin sole	1991	1.7	23.0	40.5	98.6	33
(*Pleuronectes asper*)	1992	2.3	25.0	24.2	86.7	29
	1993	2.3	25.0	24.2	86.7	29
	1994	2.3	25.0	24.2	86.7	29
	1995	2.3	25.0	24.2	86.7	29
	1996	2.3	25.0	24.2	86.7	29
	average	2.2	24.7	26.9	88.7	30
Pacific halibut	1991	2.5	25.0	52.3	99.3	39
(*Hippoglossus stenolepis*)	1992	2.3	27.0	52.3	99.3	41
	1993	2.3	27.0	52.3	99.3	41
	1994	2.3	27.0	52.3	99.3	41
	1995	2.0	27.0	64.6	99.3	33
	1996	2.3	25.5	52.3	98.4	37
	average	2.3	26.4	54.4	99.1	39
Flathead sole	1991	19.8	87.0	17.4	89.1	42
(*Hippoglossoides elassodon*)	1992	25.5	87.0	10.7	89.1	38
	1993	19.8	87.0	8.4	70.7	34
	1994	19.8	67.5	10.7	89.1	40
	1995	19.8	87.0	17.4	89.1	42
	1996	19.8	64.0	17.4	89.1	39
	average	20.8	79.9	13.7	86.0	39

catches in the LFD area was higher than in the HFD area. For Pacific halibut, the average proportion of zero catches remained approximately constant across the entire habitat area. The relative mean nonzero catch between the LFD and HFD areas varied across species, ranging from 37% to 82% (Table 4).

In each of the 24 species-year combinations, three estimates of population abundance were compared, except for flathead sole in 1992 when no samples were taken in the flathead sole HFD area (Fig. 4). In every case in which the proportion of habitat stratum-size sites to total study area sites exceeded the proportion of habitat stratum size to total study area size (Table 2), the unstratified estimate was greater than the estimate poststratified by habitat (Fig. 4). In every case that the proportion of habitat stratum sites to total study area sites was less than the proportion of habitat stratum size to total study area, the unstratified estimate was less than the estimate poststratified by habitat. Similarly, in every case that the proportion of HFD stratum sites to habitat stratum sites exceeded the proportion of HFD stratum size to habitat stratum size (Table 2), the estimate poststratified by habitat was greater than the estimate poststratified by habitat and fish density (Fig. 4). In all but two cases in which the proportion of HFD stratum sites to habitat stratum sites was less than the proportion of HFD stratum size to habitat stratum size, the estimate poststratified by habitat was less than the estimate poststratified by habitat and fish density. The two exceptions were for Pacific halibut in 1991 and 1996, where the difference between poststratified estimates was small. In 1991, the estimate poststratified by habitat was 2.9% (8116 fish) greater than the estimate poststratified by habitat and fish density; in 1996, it was 0.56% (4905 fish greater).

Table 2

A comparison of the relative number of sample sites and relative size (km^2) of the habitat area, high fish-density (HFD) area, and total study area. Comparisons include the size of the habitat area versus the size of the study area, the number of sites sampled in the habitat area versus the number sampled in the total study area, the size of the HFD area versus the size of the habitat area, and the number of sites sampled in the HFD area versus the number sampled in the habitat area.

Species	Habitat size/Total study size (km^2) All years	Habitat sites/Total sites Year						
		1991	1992	1993	1994	1995	1996	average
Rock sole (*Lepidopsetta* spp.)	0.92	0.92	1.00	1.00	0.88	1.00	1.00	0.97
Yellowfin sole (*Pleuronectes asper*)	0.66	0.78	0.87	0.63	0.60	0.80	0.80	0.75
Pacific halibut (*Hippoglossus stenolepis*)	0.62	0.73	0.93	0.58	0.52	0.80	0.80	0.73
Flathead sole (*Hippoglossoides elassodon*)	0.67	0.43	0.29	0.67	0.56	0.60	0.60	0.52

Species	High fish-density size/Habitat size (km^2) Year							High fish-density sites/Habitat sites Year						
	1991	1992	1993	1994	1995	1996	average	1991	1992	1993	1994	1995	1996	average
Rock sole (*Lepidopsetta* spp.)	0.65	0.70	0.65	0.65	0.65	0.58	0.65	0.64	0.73	0.42	0.45	0.55	0.50	0.55
Yellowfin sole (*Pleuronectes asper*)	0.58	0.51	0.51	0.51	0.51	0.51	0.52	0.76	0.46	0.47	0.40	0.50	0.50	0.52
Pacific halibut (*Hippoglossus stenolepis*)	0.72	0.76	0.76	0.76	0.61	0.69	0.72	0.69	0.86	0.79	0.62	0.63	0.54	0.69
Flathead sole (*Hippoglossoides elassodon*)	0.72	0.66	0.59	0.69	0.72	0.67	0.68	0.52	0.00	0.50	0.43	0.50	0.44	0.40

Calculations of relative efficiency among the three total abundance estimators showed increases in estimated precision with stratification (Table 5). In most cases (18 out of 24), the estimate poststratified by habitat was more precise (corresponding to a lower standard error in Fig. 5) than the unstratified estimate. Of the 16 (of 23) cases in which the precision of both poststratified estimates were greater than that of the unstratified estimate, in half the estimate poststratified by both habitat and density was more precise than the estimator poststratified by habitat alone.

Sample sizes across the survey area and in each subarea (habitat, high fish-density, and low fish-density areas) (Table 6) strongly influenced the precision of estimates. Habitat sample sizes for all species-year combinations ranged from 4 to 45 (proportion of samples taken in habitat ranged from 0.286 to 1.000); HFD sample sizes ranged from 0 to 29 (proportion of samples taken in the HFD area ranged from 0.0 to 0.8); and LFD sample sizes ranged from 4 to 16 (proportion of samples taken in the LFD area ranged from 0.125 to 0.583). Although the number of samples in both the high and low fish-density areas (Fig. 6, A and B) likely affected estimates poststratified by habitat and fish density, the number of samples in the HFD area appears to have had the primary influence on the precision of estimates. The species-year combinations for which the unstratified estimate was the most precise occurred when habitat sample sizes ranged from 4 to 22 (Fig. 7) and HFD stratum samples sizes ranged from 6 to 11 (Fig. 6A). The species-year combinations for which the estimate poststratified by habitat was the most precise occurred when habitat sample sizes ranged from 12 to 30 (Fig. 7) and when sample sizes in the HFD stratum ranged from 6 to 15 (Fig. 6A). The species-year combinations for which the estimate poststratified by habitat and fish density was most precise occurred when habitat sample sizes ranged from 15 to 45 (Fig. 7) and HFD stratum sample sizes ranged from 10 to 29 (Fig. 6A). Estimates poststratified by habitat and fish density were the most precise for all three cases in which the HFD stratum sample size was greater than 20 (corresponding to LFD stratum sample sizes ranging from 9 to 16) (Fig. 6, A and B). Both of the poststratified estimates were more precise than the unstratified estimate when habitat stratum sample sizes were greater or equal to 24 (Fig. 7) and when HFD stratum sizes were greater or equal to 12 (Fig. 6A).

Statistically significant changes in annual abundance varied among indices and species. There were significant changes in annual mean CPUE in all indices for rock sole and Pacific halibut, in two indices for yellowfin sole and in no indices for flathead sole (Table 7). Rock sole abundance was significantly greater in 1992 than all other years except 1996. Individual indices indicated that rock sole 1996 abundance was greater than that

Table 3

Annual and average proportion of zero catches in the total survey area, habitat area, high fish-density (HFD) areas and low fish-density (LFD) areas for rock sole (*Lepidopsetta* spp.), yellowfin sole (*Pleuronectes asper*), Pacific halibut (*Hippoglossus stenolepis*), and flathead sole (*Hippoglossoides elassodon*).

	Proportion of zero catches															
	Rock sole				Yellowfin sole				Pacific halibut				Flathead sole			
Year	Total survey	Habitat	HFD	LFD	Total survey	Habitat	HFD	LFD	Total survey	Habitat	HFD	LFD	Total survey	Habitat	HFD	LFD
1991	0.224	0.156	0.069	0.308	0.408	0.237	0.241	0.222	0.571	0.417	0.440	0.364	0.776	0.476	0.273	0.700
1992	0.133	0.133	0.000	0.500	0.533	0.462	0.167	0.714	0.267	0.214	0.250	0.000	0.714	0.250	—	0.250
1993	0.375	0.375	0.100	0.571	0.500	0.200	0.143	0.250	0.583	0.286	0.273	0.333	0.542	0.313	0.000	0.625
1994	0.360	0.273	0.100	0.417	0.800	0.667	0.833	0.556	0.520	0.077	0.125	0.000	0.520	0.143	0.167	0.125
1995	0.050	0.050	0.000	0.111	0.400	0.250	0.125	0.375	0.250	0.063	0.000	0.167	0.600	0.333	0.333	0.333
1995	0.133	0.133	0.067	0.214	0.433	0.292	0.250	0.333	0.333	0.167	0.154	0.182	0.733	0.556	0.250	0.800
Average	0.213	0.187	0.056	0.354	0.512	0.351	0.293	0.408	0.421	0.204	0.207	0.174	0.647	0.345	0.205	0.472

of 1992 and 1993. Tukey *post hoc* tests on the yellowfin sole all-site and habitat indices showed that 1991 yellowfin abundance was greater than that of 1994. All three indices showed that Pacific halibut abundance was greater in 1995 than in 1991 and 1993. Individual indices also indicated that Pacific halibut abundance was greater in 1995 than in 1992, 1994, and 1996.

Discussion

The Chiniak Bay multispecies survey was designed to estimate the abundance of four species with equal emphasis. Because the distribution of species varied greatly throughout the bay, what might have been an optimal stratification for individual species was compromised to develop a stratification scheme that was as effective as possible for all target species. Because we do not believe sampling was optimal for any one of the species, a poststratification method of analysis was investigated to increase the precision of abundance estimates for each species individually and to account for possible bias due to the uneven and nonrandom distribution of sampling sites over space and time.

The need for stratification and the concern about the distribution of sampling sites arise because of the varying distributions of species in the study region. Knowledge of the spatial distributions of species is important when estimating abundance from trawl surveys. A random distribution of individuals is often taken as a starting point for defining spatial distributions in ecology (Taylor et al., 1978). It is also a primary assumption for many survey sampling designs and analysis measures. The assumption of randomly distributed individuals often is not appropriate, however, because the concentration of fish varies over time and space in relation to environmental factors (Murawski and Finn, 1988; Gadomski and Caddell, 1991; Reichert and van der Veer, 1991; Norcross et al., 1999). If habitat (Fiedler and Reilly, 1994; Reilly and Fiedler, 1994) and related spatial population density distributions (Buckland and Anganuzzi, 1988) are not accounted for when calculating abundance estimates, precision can decrease and results can be seriously biased. Inaccurate results can have strong management repercussions.

In situations such as that of the present study, where the sample does not properly represent the population, poststratification is appropriate (Scheaffer et al., 1996). By comparing poststratified and unstratified estimates of abundance, we found that in every species-year combination for which the three estimates of abundance differed (Fig. 3), the poststratified estimates reduced the effect of the disproportion of samples allocated between habitat and nonhabitat areas and between high and low fish-density areas. For instance, in 1992, a disproportionately large number of samples were taken in Pacific halibut habitat (Table 2). We suspect, therefore, that the unstratified estimate of abundance was an overestimate of true population abundance. The disproportionately large number of samples taken in Pacific halibut

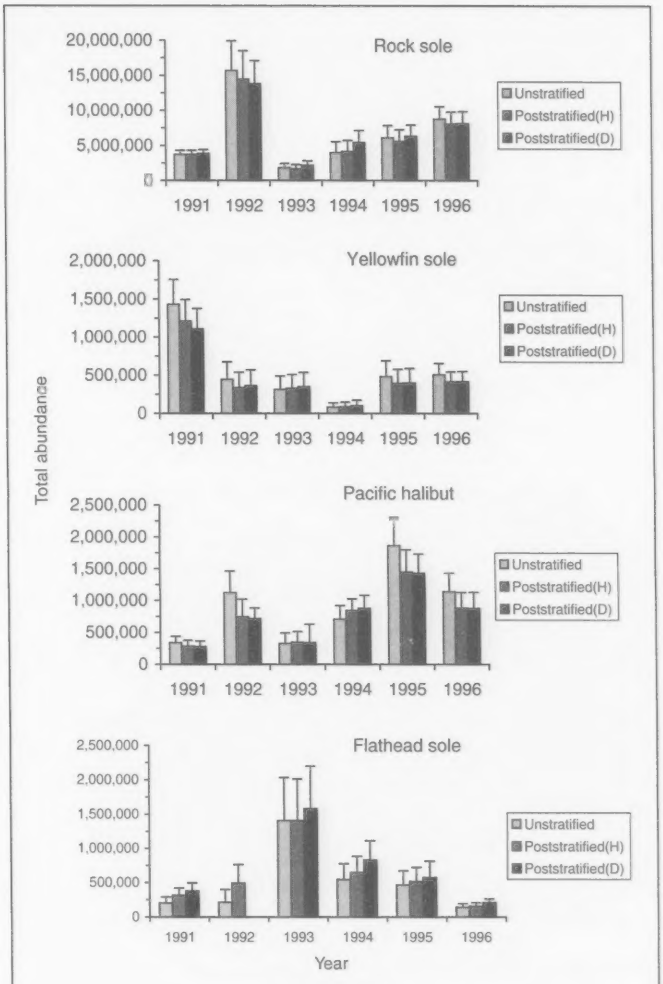

Figure 4

Three estimates of total abundance and standard error. Estimates are unstratified, poststratified by habitat (poststratified [*H*]), and poststratified by habitat and fish density (poststratified [*D*]).

habitat was adjusted by poststratifying by habitat. The estimate poststratified by habitat was less than the unstratified estimate of abundance, as we suspect the true abundance was. Poststratification by habitat and neighboring years' halibut density adjusted not only for the disproportionately large number of samples in the habitat area but also for the disproportionately large number of samples in the HFD area (Table 2). The estimate poststratified by habitat and halibut density was less than both the estimate poststratified by habitat and the unstratified estimate, as we suspect was the case for the true Pacific halibut abundance.

In 1992, the number of samples in yellowfin sole habitat was disproportionately large, but the number of samples in the HFD area was disproportionately small (Table 2). In this case, we suspect the unstratified estimate of abundance was an overestimate of true abundance because of the overabundance of samples in the habitat area. We also believe, however, that it was not a very large overestimate because of the disproportionately small number of samples in the HFD area. Poststratifying by habitat adjusted for the disproportionately large number of samples in the habitat area and produced an estimate that was less than the unstratified estimate. Poststratifying by habitat and fish density adjusted for both the disproportionately large number of samples in the habitat area and the disproportionately small number of samples in the HFD

Table 4

The mean catch per unit of effort (CPUE) of nonzero catches in the habitat, high fish-density (HFD), and low fish-density (LFD) areas and the proportion of the mean CPUE of nonzero catches in LFD and habitat areas in relation to those in the HFD area.

	Species			
	Rock sole (*Lepidopsetta* spp.)	Yellowfin sole (*Pleuronectes asper*)	Pacific halibut (*Hippoglossus stenolepis*)	Flathead sole (*Hippoglossoides elassodon*)
Habitat nonzero mean	85.3	15.6	16.8	16.0
HFD nonzero mean	105.4	20.7	17.9	20.6
LFD nonzero mean	52.2	7.6	14.7	9.5
Habitat mean/concentration mean	0.81	0.75	0.94	0.78
LFD mean/HFD mean	0.50	0.37	0.82	0.46

Table 5

Unstratified total abundance estimates (*U*), total abundance estimates poststratified by habitat (*H*), and total abundance estimates poststratified by habitat and fish density (*D*) are compared by using annual relative efficiency statistics.

Species	Relative efficiency comparison	Year					
		1991	1992	1993	1994	1995	1996
Rock sole	*H* to *U*	1.076	1.081	1.084	0.985	1.083	1.084
(*Lepidopsetta* spp.)	*D* to *H*	1.129	1.496	0.865	0.834	1.089	0.999
	D to *U*	1.214	1.618	0.937	0.821	1.179	1.084
	conclusion	*D>H>U*	*D>H>U*	*H>U>D*	*U>H>D*	*D>H>U*	*H>D>U*
Yellowfin sole	*H* to *U*	1.318	1.317	0.988	0.922	1.266	1.324
(*Pleuronectes asper*)	*D* to *H*	1.111	0.969	0.890	0.715	0.936	0.967
	D to *U*	1.465	1.277	0.880	0.659	1.186	1.280
	conclusion	*D>H>U*	*H>D>U*	*U>H>D*	*U>H>D*	*H>D>U*	*H>D>U*
Pacific halibut	*H* to *U*	1.272	1.521	1.007	1.369	1.607	1.440
(*Hippoglossus stenolepis*)	*D* to *H*	1.029	0.936	0.996	0.792	1.340	0.949
	D to *U*	1.309	1.424	1.003	1.084	2.155	1.366
	conclusion	*D>H>U*	*H>D>U*	*H>D>U*	*H>D>U*	*D>H>U*	*H>D>U*
Flathead sole	*H* to *U*	0.726	0.449	1.075	0.973	1.025	1.056
(*Hippoglossoides elassodon*)	*D* to *H*	0.786	—	0.976	0.705	0.746	0.992
	D to *U*	0.571	—	1.049	0.686	0.765	1.047
	conclusion	*U>H>D*	*U>H*	*H>D>U*	*U>H>D*	*H>U>D*	*H>D>U*

area. As a result, the estimate poststratified by habitat and fish density was greater than the estimate poststratified by habitat, but lower than the unstratified estimate. According to our results, it is unlikely that the estimates poststratified by habitat and fish density were the most representative estimates of abundance because poststratification adjusted for the disproportionate distribution of samples between areas.

Another reason to poststratify the data is to increase the precision of abundance estimates. Poststratified estimates in our study were generally more precise than unstratified estimates, given sufficient sample sizes (Table 5). Poststratification by habitat characteristics increased the precision of abundance estimates in three-quarters of all species-year combinations. This finding indicates a close link between habitat type and fish abundance and agrees with poststratification results in other studies (Pollock et al., 1994; Reilly and Fiedler, 1994). Estimates poststratified by both habitat and fish density were also generally more precise than

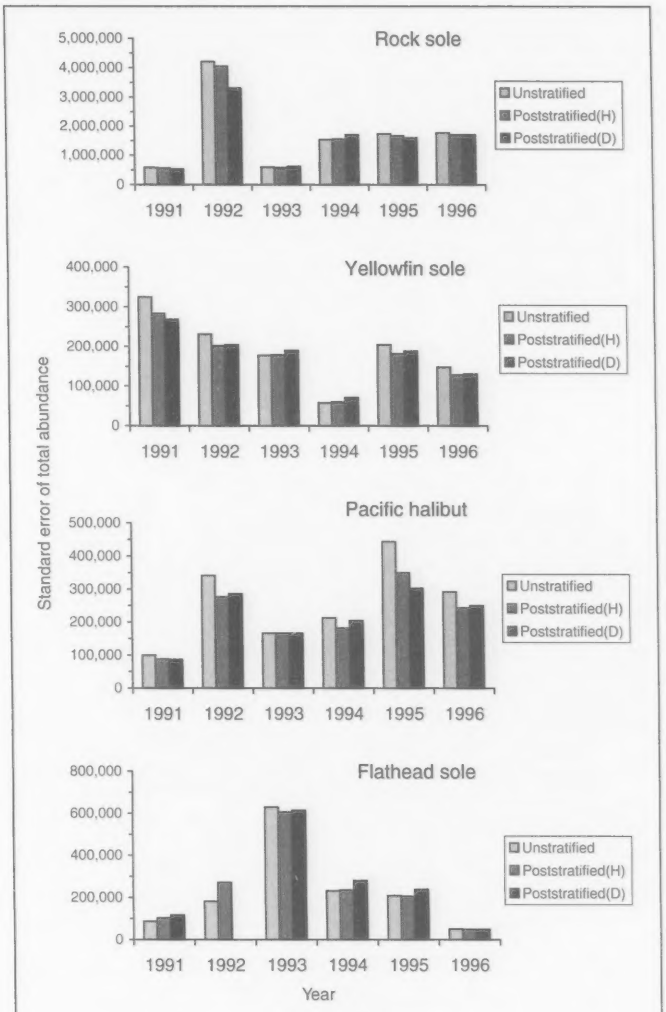

Figure 5

Three standard error estimates of annual total abundance. Standard error estimates are for the unstratified, poststratified by habitat (poststratified [H]), and poststratified by habitat and fish density (poststratified [D]) estimates.

unstratified estimates but were not consistently more precise than the estimates poststratified by habitat alone. The six cases in which estimates poststratified by habitat and fish density were the most precise show that some species have strong density gradients within habitat areas and that the incorporation of fish density information from neighboring years can be beneficial for increasing precision. Being able to predict the distribution of fish density in one year from that of neighboring years indicates annual consistency in species distribution in relation to habitat characteristics.

The present study indicates that when estimating abundance from haphazardly sampled data, the estimator poststratified by habitat is superior to the unstratified estimator regardless of sample size. The estimate poststratified by habitat was more precise than the unstratified estimate in 18 of the total 24 species-year combinations. These 18 species-year combinations occurred across nearly the full range of habitat stratum sample sizes, from 12 to 45. The six cases in which the estimate poststratified by habitat was less precise than the unstratified estimate were affected by the propor-

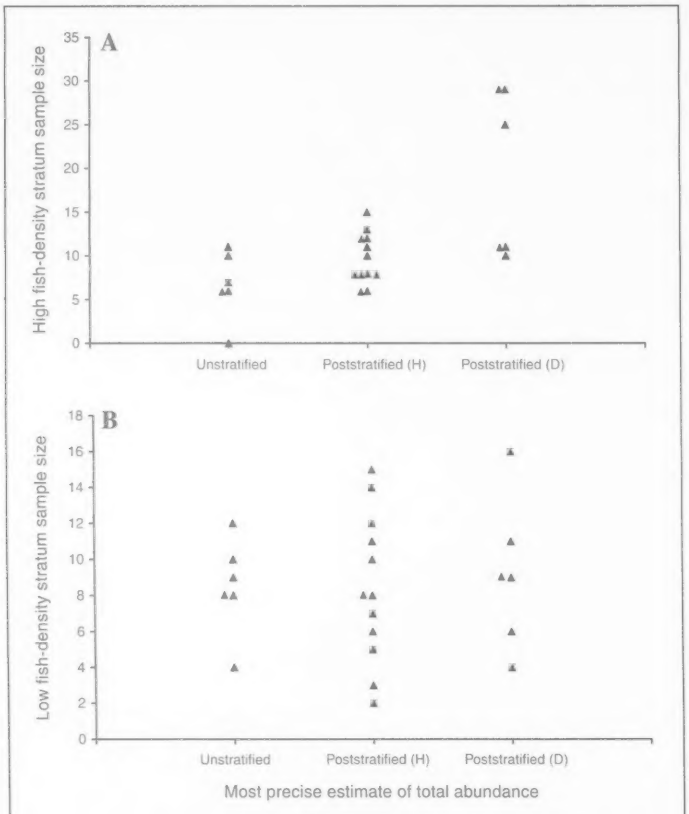

Figure 6

High and low fish-density stratum sample size in relation to the most precise estimate of total abundance. The (**A**) high fish-density and (**B**) low fish-density stratum sample size for each species-year combination is plotted in relation to the most precise estimate of total abundance—the unstratified estimate, the estimate poststratified by habitat (poststratified [*H*]), or the estimate poststratified by habitat and fish density (poststratified [*D*]).

tion of samples in unsuitable habitat. As a measure of variability, the magnitude of the variance is dependent on the magnitude of the data (Zar, 1996). Thus, the variances of trawl catches decrease as the observed means decrease (Taylor, 1953). A lower variance, therefore, does not necessarily indicate a better estimator, but instead may reflect lower population abundance. In the six cases in this study where the variance of the unstratified estimate was less than the variance of the estimate poststratified by habitat, the unstratified abundance estimate was less than the abundance estimate poststratified by habitat. The low unstratified abundance estimates in these six cases were the result of a disproportionately large number of samples in nonhabitat areas in relation to the size of the nonhabitat areas. Therefore, although the unstratified estimate was more precise, it was also likely to be an underestimate of the true abundance. Thus, we suggest that the estimate poststratified by habitat is the most desirable estimator in these situations, despite the decrease in precision in relation to the unstratified estimator.

In many cases, small sample size was likely the reason that the estimates poststratified by habitat and fish density were not the most precise of the three estimates. Poststratification produces precise estimates when the overall sample size and the sample size in each stratum are large (Scheaffer et al., 1996). In our study, the estimator poststratified by habitat and fish density was the most precise estimator of the three when sample size in the HFD stratum was 20 or greater and the sample size in the LFD stratum was 9 or greater. The number of samples in the HFD stratum appears to have had a larger influence on the precision of estimates stratified by habitat and fish density than the number of samples

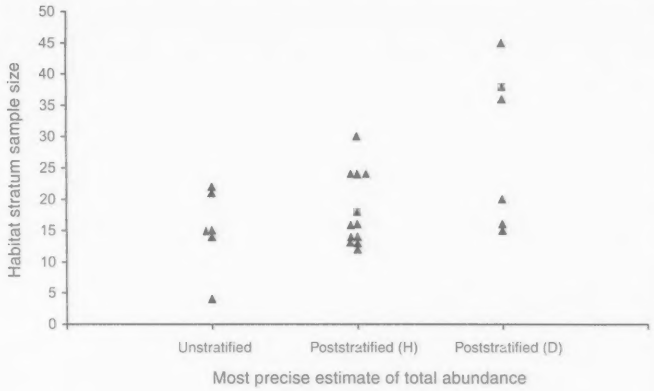

Figure 7

The habitat stratum sample size for each species-year combination is plotted in relation to the most precise estimate of total abundance—the unstratified estimate, the estimate poststratified by habitat (poststratified [*H*]), or the estimate poststratified by habitat and fish density (poststratified [*D*]).

Table 6

Annual number of tows made across all strata in habitat and nonhabitat strata, and in the high and low fish-density strata within the habitat stratum.

		Year					
Species	Stratum	1991	1992	1993	1994	1995	1996
Rock sole	all	49	15	24	25	20	30
(*Lepidopsetta* spp.)	habitat and nonhabitat	45 and 4	15 and 0	24 and 0	22 and 3	20 and 0	30 and 0
	high fish density and low fish density	29 and 16	11 and 4	10 and 14	10 and 12	11 and 9	15 and 15
Yellowfin sole	all	49	15	24	25	20	30
(*Pleuronectes asper*)	habitat and nonhabitat	38 and 11	13 and 2	15 and 9	15 and 10	16 and 4	24 and 6
	high fish density and low fish density	29 and 9	6 and 7	7 and 8	6 and 9	8 and 8	12 and 12
Pacific halibut	all	49	15	24	25	20	30
(*Hippoglossus stenolepis*)	habitat and non-habitat	36 and 13	14 and 1	14 and 10	13 and 12	16 and 4	24 and 6
	high fish density and low fish density	25 and 11	12 and 2	11 and 3	8 and 5	10 and 6	13 and 11
Flathead sole	all	49	14	24	25	20	30
(*Hippoglossoides elassodon*)	habitat and non-habitat	21 and 28	4 and 10	16 and 8	14 and 11	12 and 8	18 and 12
	high fish density and low fish density	11 and 10	0 and 4	8 and 8	6 and 8	6 and 6	8 and 10

in the LFD stratum (Fig. 6, A and B). This study supports the conclusion of Scheaffer et al. (1996) but also indicates that the sample size in the HFD stratum may have a larger influence on the precision of the resultant estimate.

As concluded in other studies (Fiedler and Reilly, 1994; Pollock et al., 1994; Reilly and Fiedler, 1994; Bernard et al., 1998), we found that poststratification can provide increased precision and decreased bias for estimates. Small stratum sample sizes, however, can make it impossible to detect heterogeneity among strata and fail to give increased precision (Powell et al., 1995; Friedland et al., 1999). The wide range of sample sizes among strata across species-year combinations exempli-

Table 7

Kruskal-Wallis test statistics for differences in annual relative abundance and, for significant Kruskal-Wallis statistics, the corresponding significant Tukey *post hoc* pairwise differences. Statistics were calculated for the all-site, habitat, and high fish-density indices.

Species	Index	Kruskal-Wallis (*indicates statistically significant difference)	Tukey *post hoc* significant differences
Rock sole (*Lepidopsetta* spp.)	All-site	P=0.0003*	1992>1991 (P<0.0006)
			1992>1993 (P<0.0001)
			1992>1994 (P<0.0009)
			1992>1995 (P<0.0124)
			1996>1993 (P<0.0301)
	Habitat	P=0.0008*	1992>1991 (P<0.0012)
			1992>1993 (P<0.0001)
			1992>1994 (P<0.0022)
			1992>1995 (P<0.0149)
			1996>1993 (P<0.0351)
	High fish density	P=0.0035*	1992>1991 (P<0.0005)
			1992>1993 (P<0.0003)
			1992>1994 (P<0.0127)
			1992>1995 (P<0.0206)
			1996>1992 (P<0.0145)
Yellowfin sole (*Pleuronectes asper*)	All-site	P=0.0033*	1991>1994 (P<0.0096)
	Habitat	P=0.0022*	1991>1994 (P<0.0374)
	High fish density	P=0.1240	
Pacific halibut (*Hippoglossus stenolepis*)	All-site	P=0.001*	1995>1991 (P<0.0013)
			1995>1993 (P<0.0012)
			1995>1994 (P<0.0359)
	Habitat	P=0.0004*	1995>1991 (P<0.0018)
			1995>1993 (P<0.0077)
	High fish density	P=0.0002*	1995>1991 (P<0.0002)
			1995>1992 (P<0.0127)
			1995>1993 (P<0.0004)
			1995>1996 (P<0.0249)
Flathead sole (*Hippoglossoides elassodon*)	All-site	P=0.1955	
	Habitat	P=0.2950	
	High fish density	P=0.5151	

fies an important drawback to using the poststratification method. Because strata criteria are unknown when sampling, it is not possible to insure that there will be sufficient samples in each poststratified stratum. When resulting sample sizes in some strata are small, poststratification may be ineffective at increasing precision. If the resulting sample size in one or more strata is one, the poststratification variance will be inestimable. If the resulting sample size in one or more strata is zero, poststratification may not be possible.

Because sample size is a limiting factor for increased precision with poststratification, there are strong implications for survey design. Many multispecies surveys are conducted by using a stratified random sampling design. There are two ways to apply poststratification to a stratified survey. First, for an unbiased estimator, each stratum of the stratified survey can be poststratified individually (Cochran, 1977). For the poststratification estimator to have increased precision beyond that of stratified random sampling, each of the original strata must have a large number of samples to allow sufficient samples in each poststratified stratum. Therefore, investigators who intend to poststratify data within a stratified random survey for unbiased estimates need to construct large strata with many samples in the original sampling design. Second, if poststratification is applied to data that were not collected under a probability sampling design, the estimator may be more precise, but may be biased. For the analysis of data that were not collected under a probability sampling

design, developing an index of relative abundance from all samples, or samples in the habitat or HFD areas, is an easy and effective way to estimate statistically significant changes in abundance among years. To determine which tows should be included in an index to effectively approximate the variations in the annual total abundance estimates, it is helpful to compare the size of the habitat area over years and to study the distribution of species density within the habitat area. The goal of creating an index should be to include the most information possible, while avoiding undue influence from the haphazard distribution of sample sites.

If the total study area is the same in each year, the choice of whether to use the all-site index should depend on whether the size of the habitat area is constant over the compared years. In this study, the defined habitat area for each species was the same over the six years compared. Therefore, for an index of relative abundance, the habitat index retained all necessary information and reduced possible bias due to the disproportionate distribution of haphazard samples between habitat and nonhabitat areas. When a temporally dependent stratification variable, such as temperature, is used to define the placement of stratum boundaries, however, the size of the habitat area may vary between years. If the annual size of the habitat area varies, some common size would need to be chosen for the relative index to approximate the annual changes in the total abundance estimates. The all-site index could be used for this purpose, but the index will be affected by any disproportionate distribution of samples between habitat and nonhabitat areas. Another possible way to do this would be to include all tows from the habitat area each year, plus as many zero catches from the nonhabitat area necessary to be proportional to the annual size of the nonhabitat area. Such an approach would not depend on actual tows in nonhabitat area but would depend on the estimated size of the habitat and nonhabitat areas and the sample size in the habitat area.

If the size of habitat area is the same in each year, the choice of whether to use the habitat index should depend on whether the distribution of species density is constant throughout the habitat area. If a species' density distribution is approximately constant across the habitat area, a haphazard distribution of sample sites should have little influence. Constructing an index from all habitat tows may then be desired to retain the largest sample size and the most information possible. Alternatively, if a species has a strong density gradient within its habitat area, a disproportionate distribution of sites in relation to the size of high and low fish-density areas may provide an unrepresentative estimate of abundance from the habitat index. In this case, if a sufficient number of samples are taken in the HFD area, constructing an index from samples within the species' HFD area alone may provide an effective index while minimizing the effect of a disproportional distribution of haphazard samples within the habitat area.

A comparison of the number of zero catches and the mean nonzero catch between the high and low fish-density areas provides information about the density distribution of species within a habitat area. The proportion of zero catches of rock sole, yellowfin sole, and flathead sole and the mean nonzero catch between high and low fish-density areas indicated density gradients within the habitat areas. Unlike these three species, the proportion of Pacific halibut zero catches was approximately the same in the HFD area as across the entire habitat area and the difference in mean nonzero catch between low and high fish-density areas was only approximately half that of the other species. Therefore, it appears that the Pacific halibut density distribution across the defined habitat area varied little compared with the other three species.

In this study, we suggest that the habitat index was the most appropriate for all four species. For each species in our study, the size of the habitat area remained the same across all six years. Thus, the habitat index eliminated the influence of disproportionately allocated samples in habitat and nonhabitat areas. For Pacific halibut, the relatively homogenous distribution of abundance across the habitat area indicates that the effect of disproportionate samples between high and low fish-density areas is small and that samples across the entire habitat area are helpful in describing annual differences in abundance. For rock sole, yellowfin sole, and flathead sole, the difference in the proportion of zero catches and nonzero mean abundance between the high and low fish-density areas was considerable. As a result, differences in annual abundance suggested by the habitat index may be affected by the inconsistent disproportion of samples between high and low fish-density areas over years. Although it would be preferable to use the HFD index in these cases, annual sample sizes in the HFD area were so small that we recommend the habitat index instead. Recognizing that the habitat index will not account for the annual disproportion of samples between the high and low fish-density areas, we used the comparison of the size and the number of samples taken in high and low fish-density areas to flag differences in annual index abundance estimates that might be over- or underestimates. If this method is applied in a management context, the levels of the factors describing the density distribution of the species (i.e., difference in the percent of zero catches and the percent difference in mean nonzero catch between years) can be set as criteria and kept constant over years to eliminate subjectivity between years or between species. For example, if the percent of zero catches in high and low fish-density regions differ by 40% and the mean nonzero catch in the HFD area is 30% greater than that in the LFD area, the HFD index should be used. Otherwise, the habitat index should be used.

For many surveys, identifying habitat and fish-density areas for poststratification and index construction is possible with currently available information. The estimation methods used in the present study can be applied to any survey for which abundance and environmental measurements are available for each sampled site and the environmental measurements are related to species

abundance in a consistent way. For example, the NMFS Bering Sea trawl survey includes measurements of depth and surface and bottom temperatures at all trawl sites (Goddard and Walters[4]) that could be used for poststratification. Similarly, the Pacific West Coast trawl survey includes measurements of surface and bottom temperature and salinity at all stations (Lauth et al.[5]) that could be used. Poststratification allows for use of a wide range of stratification variables, including temporally dependent variables that are not available before sampling is complete, e.g., temperature and salinity.

For surveys where habitat information is not collected at trawl sites, habitat information from other sources can be paired with fish distribution information after collections have been made. For instance, when habitat information is available, but has not been collected at each site, spatial statistics can be used to krige the habitat information over the study area and to predict the specific habitat data value at the sampling sites. If there is a consistent relationship between species abundance and the habitat variable, the catch and habitat data paired at sample sites can then be used to identify areas of suitable habitat and areas of high fish density within suitable habitat. How well habitat and HFD areas are estimated will depend on the number and distribution of habitat measurements, the contouring algorithms used, and the estimates of areas within contours. Even if species are not distributed in direct response to particular environmental characteristics, the characteristics may serve as proxies for effects that are more difficult to measure (Perry and Smith, 1994). Once habitat and HFD areas are identified, poststratification can be conducted for total abundance estimates, and statistically significant changes between years can be assessed with an index of relative abundance. These methods could yield more accurate estimates of abundance for use by managers. The goal of most sampling plans is to provide statistical estimates with the smallest possible confidence limits at the lowest cost (Krebs, 1989). Thus, being able to use data collected independently of a survey should be appealing.

The NRC (2000) recommends using data from commercial or sportfishing vessels in scientific assessments of abundance. A primary difficulty in using commercial fisheries data for scientific estimates of abundance is that the data do not represent random samples of the fish population. As a result, commercial fisheries data present a biased perspective of the population that may change over time and may not correlate well with actual fish abundance (NRC, 2000). Although commercial fishery-dependent data may provide biased estimates of abundance, fishery-dependent data also provide large sample sizes and a wide range of information not available from other sources. For example, commercial and sportfishing data often provide broader geographic and temporal coverage. Poststratification of haphazard data from commercial and sportfishing sources may be one way to reduce inherent bias and provide useable scientific information. For instance, Buckland and Anganuzzi (1988) described how data collected on commercial tuna fishing vessels can be used to estimate dolphin abundance when survey data are not sufficient. The data collection sites were not randomly selected. Instead, the sampling sites were directly related to dolphin sightings, because dolphins and tuna schools are often closely associated. As a result, areas of high dolphin density corresponded with areas of high fishing effort. Poststratification was used to decrease the bias resulting from nonrandom distribution of both search effort and dolphin schools. A second example is a retrospective study that combined survey and commercial fishing data. In this study (Halliday[6]), 1958–60 poststratified survey data were used to develop a relationship between the survey abundance of the 1954–1959 year classes and their abundance estimates from commercial fishery data. This relationship was then used, along with 1969 survey data, to predict the size of the 1966–68 year classes. The same process was used to predict the size of later year classes with later years of survey data.

Poststratification also facilitates the use of a single data set for multiple objectives. Collecting data is costly and many data sets are collected and analyzed for a single objective and then not used again. Although it is preferable to use data for multiple objectives, it can be difficult to meet statistical assumptions when the data are re-used for a different purpose. For example, a multispecies survey may be stratified according to the distribution of one or more of the most commercially valuable species collected. An example is the stratification of Pacific west coast bottom trawl surveys in 1980, 1983, and 1986, which were focused to improve the precision of canary and yellowtail rockfish abundance estimates (Weinberg et al.[2]). If the stratification used was not effective for decreasing the variance of abundance estimates for other species, treating the data as if they were haphazardly collected, recognizing that the estimator may be biased, and poststratifying the data by habitat variables that are closely related to the

[4] Goddard, P., and G. Walters. 1998. 1995 bottom trawl survey of the eastern Bering Sea continental shelf. AFSC Processed Report 98-08, 170 p. Resource Assessment and Conservation Engineering Division, Alaska Fisheries Science Center, NMFS, NOAA, 7600 Sand Point Way N.E., Seattle, Washington, 98115.

[5] Lauth, R. R., M. E. Wilkins, and P.A. Raymore Jr. 1997. Results of trawl surveys of groundfish resources of the West Coast upper continental slope from 1989 to 1993. NOAA Tech. Memo. NMFS-AFSC-79, 342 p. National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, Virginia 22161.

[6] Halliday, R. G. 1970. 4T-V-W haddock: recruitment and stock abundance in 1970–72. ICNAF Res. Doc 70/75, 12 p. Approved for citation by Tissa Amaratunga, Deputy Executive Secretary, Northwest Atlantic Fisheries Organization. [Available from the Secretariat Library, Northwest Atlantic Fisheries Organization, 2 Morris Drive, Burnside Industrial Park, Dartmouth, Nova Scotia, Canada, B3B 1K8.]

distribution of the other species may be a beneficial way to make multiple uses of the data. Although the poststratified estimator may be biased, poststratification may provide large gains in precision and a decrease in bias in relation to an unstratified estimator. Large increases in precision may be worth the acceptance of some bias.

Multispecies surveys are often not optimal for estimating the abundance of individual species but are often necessary because of limited time and financial resources. As a result, researchers need to explore alternative sampling and analysis designs to increase the precision of individual species abundance estimates (NRC, 2000). Poststratification is a method that can be applied to any number of species by using a wide range of habitat and other variables that can be stratified. Because of the dramatic increase in habitat information that is likely to be collected in response to the expanded emphasis in the Magnuson-Stevens Act (NRC, 2000) and because of the adaptability of poststratification for handling a multitude of types of data sets, the method of poststratification may provide increased usefulness for scientific researchers.

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Length at maturity in three pelagic sharks (*Lamna nasus, Isurus oxyrinchus,* and *Prionace glauca*) from New Zealand

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Abstract—Reproductive data collected from porbeagle, shortfin mako, and blue sharks caught around New Zealand were used to estimate the median length at maturity. Data on clasper development, presence or absence of spermatophores or spermatozeugmata, uterus width, and pregnancy were collected by observers aboard tuna longline vessels. Direct maturity estimates were made for smaller numbers of sharks sampled at recreational fishing competitions. Some data sets were sparse, particularly over the vital maturation length range, but the availability of multiple indicators of maturity made it possible to develop estimates for both sexes of all three species.

Porbeagle shark males matured at 140–150 cm fork length and females at about 170–180 cm. New Zealand porbeagles therefore mature at shorter lengths than they do in the North Atlantic Ocean. Shortfin mako males matured at 180–185 cm and females at 275–285 cm. Blue shark males matured at about 190–195 cm and females at 170–190 cm; however these estimates were hampered by small sample sizes, difficulty obtaining representative samples from a population segregated by sex and maturity stage, and maturation that occurred over a wide length range. It is not yet clear whether regional differences in median maturity exist for shortfin mako and blue sharks.

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The attainment of sexual maturity in sharks is a major developmental milestone which has a large impact on their distribution, behavior, and biology. Immature sharks often associate with each other regardless of sex, but after maturity sexual segregation is the norm. Mature males and females may come together only to mate, resulting in movements that may range from small-scale aggregation of dispersed individuals to long-range migrations over thousands of kilometers.

The process of maturation, and the subsequent need to channel energy into reproduction, affect the growth rate of at least some shark species. Immature male and female porbeagles grow at the same rate, and the growth rate of both sexes slows at maturity; however females mature at a greater age than males and therefore their period of fast immature growth lasts longer and they grow larger than males (Natanson et al., 2002).

The maximum reproductive lifespan of a shark species is the time elapsed between the age at maturity and the maximum age. In conjunction with the duration of the reproductive cycle, the reproductive lifespan determines the maximum number of litters a female shark can produce in her lifetime. Population modeling indicates that shark species that mature at a young age have a greater capacity to recover from exploitation than sharks that mature later (Smith et al., 1998). Thus age at maturity is a crucial factor influencing the productivity of a species.

Age at maturity can be estimated directly from paired age-and-maturity estimates taken from the same shark, but often such data are not available, or are too few to provide precise estimates. Consequently it is often necessary to estimate age at maturity indirectly from length at maturity and a growth curve.

In the present study we estimate the length at maturity for three species of large pelagic sharks in New Zealand waters: porbeagle (*Lamna nasus* (Bonnaterre, 1788)), shortfin mako (*Isurus oxyrinchus* Rafinesque, 1810), and blue (*Prionace glauca* (Linnaeus, 1758)) sharks. These species are commonly caught by tuna longliners fishing around New Zealand (Francis et al., 2001). Longline fishing effort declined from a high of over 25 million hooks per year in the early 1980s, to a low of 2–4 million hooks in 1995–98, largely because of a reduction in the number of foreign licensed vessels (Francis et al., 2001). Since then, the domestic longline fleet has expanded, and fishing effort exceeded 10 million hooks in 2001–02 (Ayers et al., 2004). Because of concern over the sustainability of the catches of both target and nontarget species in this fishery, the New Zealand Ministry

of Fisheries introduced individual transferable quotas for a number of pelagic species, including the three sharks, in October 2004.

Despite the panglobal distributions of porbeagle, shortfin mako, and blue sharks, and their importance in the catches of pelagic longline fisheries worldwide, comparatively little effort has been devoted to estimating their length (or age) at maturity. In the northwest Atlantic Ocean, the length at maturity of male and female porbeagles has been well determined (Jensen et al., 2002), but preliminary data from the southwest Pacific Ocean indicate that females mature at a much smaller length there (Francis and Stevens, 2000). Mollet et al. (2000) found significant differences in the length at maturity of female shortfin makos between the Northern and Southern hemispheres; however there is little information on the length at maturity of male makos (Stevens, 1983). Blue sharks have been studied in a number of regions worldwide (Pratt, 1979; Stevens, 1984; Hazin et al., 1994; Nakano, 1994; Castro and Mejuto, 1995), but size and sex segregation have made it difficult to obtain representative samples of both sexes from which to determine length at maturity.

In the southwest Pacific Ocean, estimates of length at maturity are lacking or uncertain for at least one sex of all three species. Although all species make long distance movements, and presumably have wide-ranging stocks, the interhemispheric differences in length at maturity reported for female porbeagles and shortfin makos indicate that it is not safe to transfer estimates from one region to another. The aim of the present study is to develop region-specific estimates of length at maturity for male and female porbeagle, blue and shortfin mako sharks, and to determine whether this parameter varies globally. These results will contribute to efforts to determine the productivity and stock status of pelagic sharks in New Zealand waters.

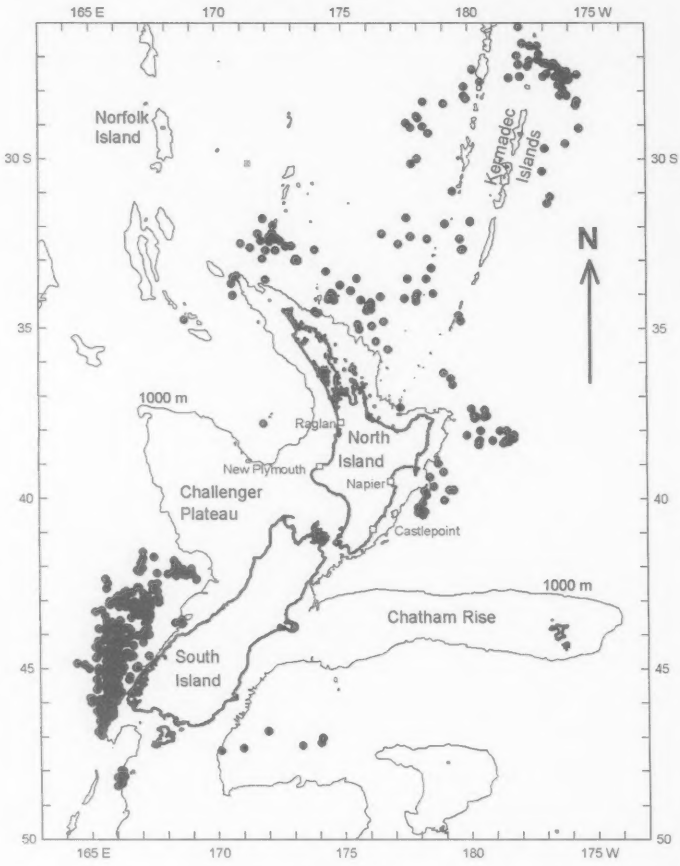

Figure 1

Start-of-set positions of tuna longline sets during which observers sampled porbeagle (*Lamna nasus*), shortfin mako (*Isurus oxyrinchus*), and blue (*Prionace glauca*) sharks. Also shown are the North Island ports where sharks landed during fishing competitions were sampled.

Materials and methods

Data sources

Reproductive data were collected from two main sources. The first consisted of sharks sampled by the authors at recreational fishing competitions, or occasionally sharks retained by commercial fisheries or research vessels. The second source consisted of data and occasionally embryos and female reproductive tracts collected by Ministry of Fisheries observers aboard commercial tuna longline vessels (Fig. 1). Sharks obtained from fishing competitions provided the opportunity to measure a wide range of reproductive parameters on relatively small samples, whereas sharks observed on tuna longline vessels provided large samples but limited reproductive data.

Sharks obtained from fishing competitions

Competition sharks consisted mainly of makos and blue sharks sampled at fishing competitions around the North Island (Fig. 1). Most sharks were sampled from the Hawke Bay competition held annually in February or March from the port of Napier. Other significant competitions were sampled at Castlepoint, Raglan, and New Plymouth. All except two of the competition sharks were collected in summer (January–March) and samples

spanned the period from 1986 to 2004. In the early years, only data on length, sex, weight, and maturity were collected. In later years, detailed reproductive data were also collected. The following length measurements were made as point-to-point straight line distances to the whole centimeter below actual length:

Total length (TL_{nat}):	tip of snout to a perpendicular dropped from the tip of tail to the midline (with the tail in the natural position);
Total length (TL_{flex}):	tip of snout to tip of tail (with the tail flexed towards the midline to provide maximum extension);
Fork length (FL):	tip of snout to fork in the tail;
Precaudal length (PCL):	tip of snout to the upper precaudal pit (mako and porbeagle sharks) or the origin of the upper caudal lobe (blue sharks).

Total weight was measured on accurate scales provided at the fishing competitions, on research vessels, or in commercial fish processing sheds.

In males, inner clasper length was measured between the anterior margin of the cloaca and the posterior clasper tip, and expressed as a percentage of fork length:

$$\text{Clasper length index (CLI)} = 100\ (\textit{clasper length}/\textit{FL}).$$

The degree of clasper calcification and development was determined and included an assessment of whether the terminal cartilages could be splayed open, whether a spur was present and erupted, and whether the entire clasper could be rotated. In some males sampled in later years, the degree of development of the testes, epididymis, and ampulla at the posterior end of the epididymis was also recorded, and occasionally testes were weighed and measured (following dissection from the epigonal organ if necessary). The presence or absence of spermatophores or spermatozeugmata in the ampulla epididymis was noted. (Spermatophores are masses of encapsulated sperm, and they are found in porbeagle and mako sharks; spermatozeugmata are unencapsulated masses of naked sperm that are found in blue sharks [Pratt and Tanaka, 1994]).

In females, the reproductive system was examined, and in later years a number of measurements were taken. Uterus width was measured near the middle of the body cavity and expressed as a percentage of fork length:

$$\text{Uterine width index (UWI)} = 100\ (\textit{uterus width}/\textit{FL}).$$

The maximum diameter of ova, where they were sufficiently developed to be visible in the ovary, was recorded, and the diameter of the oviducal gland was measured. Ovarian dimensions and weight were measured after dissection (if necessary) from the epigonal organ. Any contents of the uteri were noted; embryos were measured and sex was determined. The presence or absence of a hymen (cloacal membrane occluding the vaginal opening) was recorded.

For both males and females, a direct assessment of maturity (hereafter called direct maturity) was made by using all the available reproductive data. A three-stage classification scheme was used: immature, maturing, and mature. Mature sharks were defined as those in which the reproductive system was judged to be fully functional and capable of delivering reproductive products. For analysis purposes, maturing sharks were grouped with immature sharks.

Sharks sampled by observers

Observers sampled tuna longline sets from around the New Zealand region (Fig. 1). Data from blue and mako sharks were obtained throughout the sampled area, but porbeagles came mainly from the southwestern South Island. Most sharks were sampled in autumn–winter (April–July) over the period 2001–2003. The "standard" length measurement for sharks was FL, but frequently observers also recorded TL_{nat} or PCL.

Observers were provided with instructions and photographs indicating the reproductive data they needed to collect, but they were not provided with any practical training. The main data they collected were the following: inner clasper lengths, presence or absence of spermatophores or spermatozeugmata in the ampulla epididymis (for males); uterus width, maximum ovum diameter, and whether the shark was pregnant or not (for females).

Examination of observer pregnancy records for blue sharks indicated numerous probable errors: uterus widths from sharks scored as pregnant were frequently less than 18 mm, which seems implausible considering that ova are ovulated at about 18 mm, and pregnant sharks are unlikely to have such small uteri (Pratt, 1979; Natanson[1]). This problem was apparent for several observers, some of whom were very experienced (although they had no previous experience examining shark reproductive systems). We suspect that they may have scored some female blue sharks as pregnant if the ovary contained large yolky ova (this problem did not occur for mako and porbeagle sharks, which have much smaller ovarian ova). We therefore used observer blue shark pregnancy records only if they were supported by appropriate comments on the data sheet (e.g., mention of embryos or ovulated eggs in uteri), or if the observers retained embryos or intact uteri for us to examine.

Observers did not assess direct maturity; therefore we were unable to derive direct maturity ogives for observer sharks.

[1] Natanson, L. 2004. Unpubl. data. National Marine Fisheries Service, 28 Tarzwell Drive, Narragansett, Rhode Island 02882-1152.

Table 1

Regression equations used to convert shark lengths reported in the literature. r^2=the coefficient of determination; n=sample size. Measurement method acronyms are defined in the "Materials and methods" section, except that CFL = curved fork length and CTL = curved total length (both measured over the curve of the body).

Species	Regression equation	r^2	n	Data range (cm)	Source
Porbeagle	$FL = -6.943 + 0.893\ TL_{nat}$	0.997	103	61–181 FL	This study
	$FL = 0.90 + 0.95\ CFL$	0.997	172	83–253 FL	S. Campana[1]
Mako	$CFL = -1.7101 + 0.9286\ CTL$	0.997	199	65–338 CFL	Kohler et al., 1995
	$FL = 0.973 + 0.968\ CFL$	0.999	30	113–287 FL	This study
	$FL = 0.766 + 1.100\ PCL$	0.997	999	61–346 FL	This study
	$FL = 0.821 + 0.911\ TL_{nat}$	0.993	399	70–346 FL	This study
Blue	$FL = -0.90 + 0.98\ CFL$	0.99	789	123–286 FL	S. Campana[1]
	$FL = -1.615 + 0.838\ TL_{nat}$	0.990	273	50–270 FL	This study
	$FL = 0.745 + 1.092\ PCL$	0.998	12,657	34–326 FL	This study

[1] Refers to footnote 2 in the general text.

Data analysis

For each shark species and sex, we were interested in determining the length at which 50% of the individuals in a population reached full sexual maturity. That length is the median length at maturity, hereafter referred to as "median maturity."

Many shark species show abrupt transitions in the sizes of reproductive organs near length at maturity. To locate such transitions in clasper length, we fitted "split" linear regressions to CLI data plotted against FL. Split regressions consist of two simple linear regressions fitted to different nonoverlapping data ranges that meet at a point called the breakpoint (Kovác et al., 1999). A split regression has the form

$$\begin{aligned} \text{CLI} &= f(FL - p) + h \text{ for } FL < p \\ \text{CLI} &= g(FL - p) + h \text{ for } FL \geq p, \end{aligned}$$

where f and g are slope parameters for the two limbs of the regression, and h and p are the y-axis and x-axis coordinates of the breakpoint, respectively. The parameters f, g, h, and p were estimated by least squares by using the curve fitting routine in the Sigmaplot statistical and graphing package (Sigmaplot, vers. 9.01, Systat Software Inc., Richmond, CA). The length at the breakpoint was corrected for downward rounding of FL by adding 0.5 cm.

Maturity ogives were fitted to the direct maturity data separately by sex by using probit analysis (Pearson and Hartley, 1962). The analyses were performed on individual FL measurements, but we also calculated the proportions of mature individuals in 10-cm length classes to illustrate the trends. Probit analysis assumes that the length at which a randomly selected fish reaches maturity is normally distributed. Two parameters, the mean and standard deviation of the normal distribution, were fitted. Each maturity ogive is the cumulative distribution function for the associated normal distribution. The probit function was fitted by maximum likelihood, and 95% confidence limits were estimated by the bootstrap method. The mean of the normal distribution is an estimate of the median maturity, and it was corrected for downward rounding of FL by adding 0.5 cm.

All shark length measurements provided in the present study are FL, unless otherwise stated. For comparison with our results, we converted measurements from the literature to FL where necessary using the regression equations in Table 1. Literature reports of total length were assumed to be TL_{nat} unless otherwise stated. Scientists working on sharks in the northeastern United States, and eastern Canada have typically measured lengths over the curve of the body rather than as straight line distances (Natanson[1]; Campana[2]; Pratt[3]), notwithstanding some published statements to the contrary (Pratt, 1979; Kohler et al., 1995).

Results

Porbeagle shark

In male porbeagles, CLI showed two strong inflection points: the first at about 110 cm, and the second, esti-

[2] Campana, S. E. 2004. Personal commun. Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, Nova Scotia, Canada B2Y 4A2.

[3] Pratt, H. L. 2004. Personal commun. Mote Marine Laboratory, 24244 Overseas Highway, Summerland Key, FL 33042.

mated by split linear regression fitted to sharks longer than 110 cm, at 142.7 cm (95% confidence interval (CI) 140.7–144.7 cm) (Fig. 2). Thus rapid elongation of the claspers began at about 110 cm and was completed by 143 cm. Spermatophores first appeared in the posterior reproductive tract at 135 cm and by about 152 cm 50% of males contained spermatophores. The percentage of sharks with spermatophores peaked at 165 cm (82% of males) and then declined to about 50%, although sample sizes were small in the longer length groups (Table 2).

In females, UWI began increasing at a length of about 145 cm, but many larger, nonpregnant sharks showed no expansion of the uteri (Fig. 3). Three females with UWI of about 4–5% were postpartum, and two with UWI about 11% and one with UWI of about 4% were pregnant. Pregnant females measured 167–202 cm (mean 184 cm, n=55). Of 19 females longer than 175 cm that were scored by observers for pregnancy, 10 (53%) were pregnant, two (11%) were postpartum, and seven (37%) were resting (or possibly immature).

Apart from a 185-cm pregnant female, all whole porbeagles examined by us were immature; therefore no attempt was made to estimate maturity directly.

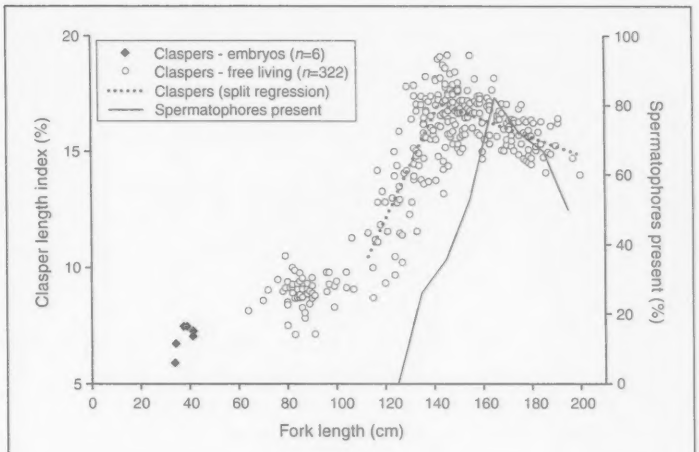

Figure 2

Maturation of male porbeagle sharks (*Lamna nasus*): variation in clasper development and presence of spermatophores in the reproductive tract.

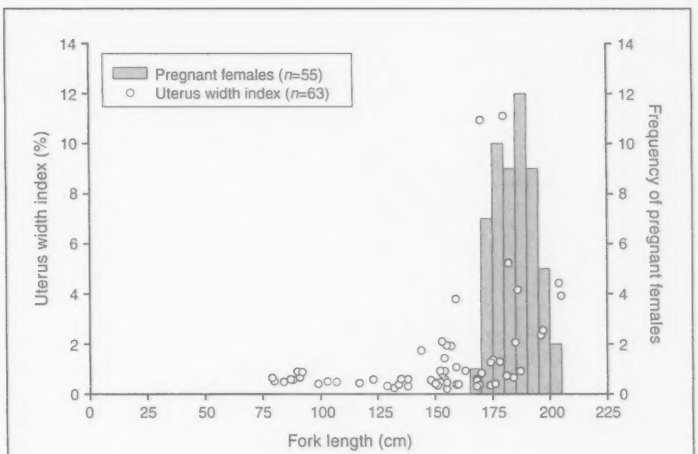

Figure 3

Maturation of female porbeagle sharks (*Lamna nasus*): variation in uterus width index, and length-frequency distribution of pregnant females.

Shortfin mako shark

CLI showed two strong inflection points in male makos; the first at about 140 cm and the second (estimated by split linear regression) at 185.1 cm (CI 182.5–187.7 cm) (Fig. 4). The smallest male with spermatophores was 136 cm, but this measurement was an outlier and may have been an error; the next smallest was 156 cm. Fifty percent of males contained spermatophores by 178 cm, and 100% by about 235 cm. Sample sizes were reasonable over the transition range but small above 230 cm (Table 2).

Male makos examined by us showed little overlap in length between immature and mature sharks (Fig. 4), but sample sizes were small in all length classes (Table 2). The smallest mature male was 182 cm and the largest immature male was 183 cm long. The median maturity estimated by probit analysis was 182.9 cm (CI 180.7–185.1 cm) (Fig. 4).

In females, UWI began increasing at a length of about 275 cm, and all larger sharks had expanded uteri (Fig. 5). Only one pregnant female mako has been recorded from New Zealand waters, and it was 290 cm FL (Duffy and Francis, 2001); no uterus width measurement was available for that shark. The remaining makos over 275 cm were either postpartum or resting. The maximum ovum diameter began increasing in sharks longer than 250 cm (in shorter sharks, ova were barely visible or were invisible) (Fig. 6). The diameter of the oviducal gland increased abruptly between 250 and 270 cm, but ovary dimensions showed no abrupt change in size (Fig. 6).

Median maturity was estimated directly from a sample of 88 females (Table 3). The smallest mature female

Table 2

Sample sizes by 10-cm length class for the assessment of maturity in male porbeagle, mako, and blue sharks.

Length class midpoint (cm)	Porbeagle shark	Shortfin mako shark		Blue shark	
	Spermatophores	Spermatophores	Direct maturity	Spermatozeugmata	Direct maturity
45	0	0	0	0	1
55	0	0	0	0	3
65	0	0	0	0	2
75	2	0	0	0	0
85	15	0	1	1	0
95	4	0	0	2	1
105	0	3	1	2	0
115	2	3	3	0	0
125	8	3	0	1	0
135	23	4	8	1	0
145	28	4	11	3	1
155	30	9	4	6	5
165	17	10	6	13	3
175	18	19	3	4	6
185	6	16	7	20	4
195	4	15	1	21	6
205	1	27	1	18	6
215	0	19	4	15	4
225	0	14	1	12	1
235	0	8	0	20	8
245	0	5	1	26	2
255	0	3	0	19	1
265	0	0	0	11	1
275	0	1	0	6	2
285	0	0	0	2	0
295	0	0	0	1	1
Total	158	163	52	204	58

Table 3

Sample sizes by 10-cm length class for the assessment of maturity in female mako and blue sharks.

Length class midpoint (cm)	Shortfin mako shark	Blue shark	Length class midpoint (cm)	Shortfin mako shark	Blue shark
	Direct maturity	Direct maturity		Direct maturity	Direct maturity
55	0	6	215	10	2
65	0	3	225	10	0
75	0	1	235	6	0
85	0	2	245	9	0
95	0	0	255	4	0
105	0	0	265	3	0
115	2	0	275	1	0
125	2	0	285	2	0
135	2	0	295	3	0
145	10	1	305	1	0
155	6	1	315	0	0
165	3	0	325	3	0
175	1	3	335	2	0
185	3	5	345	1	0
195	0	0	Total	88	26
205	4	2			

was 274 cm and the longest immature female was 300 cm. Median maturity was estimated by probit analysis to be 280.1 cm (CI 267.5–292.9 cm), but sample sizes were very small over the transitional range (Fig 5). The nonoverlap of the CIs between males and females showed that median maturity differs significantly between the sexes.

Blue shark

The relationship between CLI and FL was essentially linear in blue sharks, and no apparent inflections were evident (Fig. 7). The smallest male with spermatozeugmata was 164 cm; 50% of males contained spermatozeugmata by 194 cm, and 100% by about 260 cm.

Samples of male blue sharks examined by us were small (Table 2). Maturation occurred over a wide length range: the smallest mature male was 157 cm and the largest immature male was 237 cm long. The direct estimate of median maturity was correspondingly variable (192.1 cm, CI 178.1–206.3 cm) (Fig. 7).

The UWI increased abruptly above about 170 cm in some sharks, all of which were pregnant (Fig. 8). Other nonpregnant sharks up to about 220 cm FL, which were presumably subadults, had UWIs less than 2%. Pregnant females ranged from 166 to 252 cm (mean 203 cm) (Fig. 8).

Only 26 females were available for direct maturity estimation (Table 3). The smallest recorded mature female was 142 cm, but this seems exceptional; the next smallest was 172 cm. The longest immature female was 185 cm. The number of sharks in the maturation length range was inadequate for determining median maturity (Table 3), although we have shown the probit analysis ogive in Figure 8.

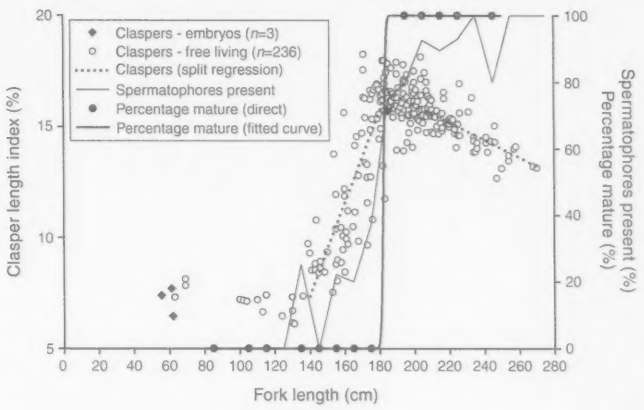

Figure 4

Maturation of male shortfin mako sharks (*Isurus oxyrinchus*): variation in clasper development, presence of spermatophores in the reproductive tract, and direct maturity estimation determined from a suite of maturity indicators.

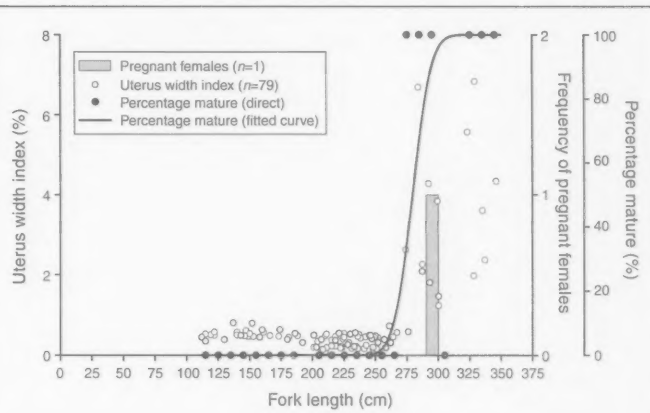

Figure 5

Maturation of female shortfin mako sharks (*Isurus oxyrinchus*): variation in uterus width index, and direct maturity estimation from a suite of maturity indicators. The only pregnant female recorded from New Zealand waters is also indicated.

Discussion

Maturity estimates

To be sexually mature, a male shark must be able to produce viable sperm and have the means to deliver them to a female. Similarly, females must be able to produce viable eggs and nourish the developing embryos through to parturition. An assessment of the degree of development of all parts of the reproductive system and the presence or absence of reproductive products is the best way to determine sexual maturity. We used this approach to score the maturity status of individual sharks and thereby derive direct median maturity estimates. However, the sample sizes available for this approach were sometimes small, and confidence limits ranged from unrealistically low (because of lack of overlap of immature and mature sharks) to high; therefore it was not possible to rely entirely on these estimates.

We supplemented our direct maturity estimates with measurements or assessments (made by observers) of some key components and products of the reproductive

system. The presence or absence of spermatophores or spermatozeugmata is a good indicator of the ability of a male to produce viable sperm, but it is not infallible: such structures sometimes lack viable sperm (Pratt and Tanaka, 1994). Furthermore, male reproductive products may not be present year-round: blue sharks appear to have a seasonal cycle of spermatozeugmata production in the western central Atlantic (Hazin et al., 1994), although Pratt (1979) found no evidence of a cycle in the western North Atlantic. Thus the presence of spermatophores and spermatozeugmata does not necessarily confirm reproductive viability, and their absence does not confirm immaturity. Similarly, fully calcified claspers that can be rotated, splayed open, and possess an anchoring mechanism may confer an ability to mate, but they do not necessarily confirm an ability to deliver viable products; however the lack of fully developed claspers presumably does prevent successful copulation.

In the present study, either the length at which clasper development was completed in half the male population, or the length at which 50% of males possessed spermatophores or spermatozeugmata, whichever was higher, provided an estimate of the lower bound of the median maturity. The actual median maturity may be higher than this estimate if some males had reproductive products that lacked viable sperm, or if some other feature of the reproductive system (e.g., the siphon system) was insufficiently developed to enable delivery of sperm to the female.

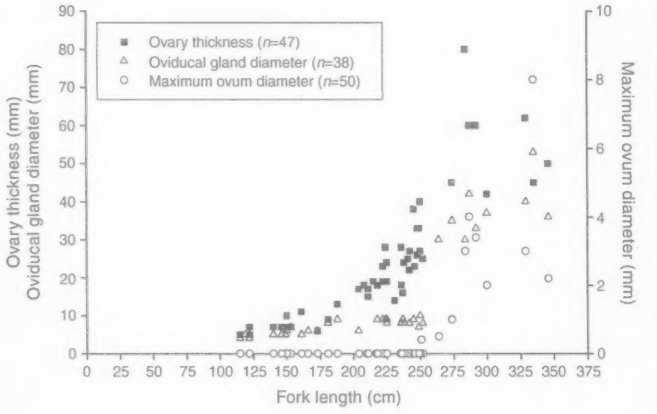

Figure 6

Maturation of female shortfin mako sharks (*Isurus oxyrinchus*): relationship between fork length and ovary thickness, oviducal gland diameter, and maximum ovum diameter.

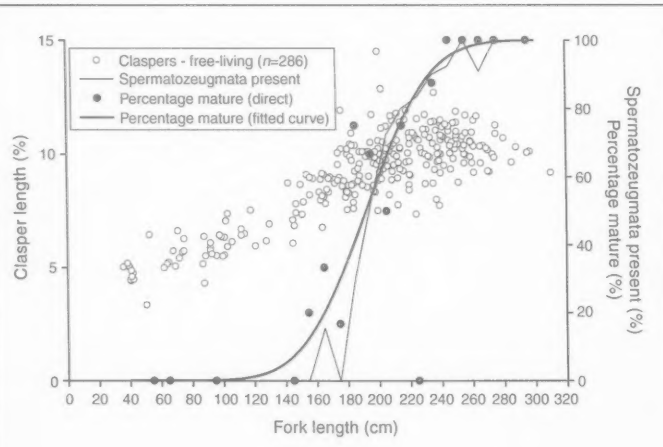

Figure 7

Maturation of male blue sharks (*Prionace glauca*): variation in clasper development, presence of spermatozeugmata in the reproductive tract, and direct maturity estimation from a suite of maturity indicators.

An analogous argument applies to female sharks. Full development of the uterus and oviducal gland, and production of vitellogenic ovarian ova, are all required for successful reproduction. Expansion of the uterus, as measured here by UWI, may not be a sufficient condition by itself. Thus the length at which half the female population had expanded uteri places a lower bound on the median maturity.

The smallest length at which females were pregnant, and the length-frequency distributions of pregnant females, are not by themselves good indicators of median maturity. A better indicator would be the length at which half the females in a population first become pregnant, but this is impossible to determine. Furthermore, pregnancy estimates could be confounded by unrepresentative sampling of a population that may be segregated by reproductive status and by nonparticipation of some females during breeding because they are "resting" between pregnancies. Nevertheless, pregnancy absolutely confirms maturity; therefore it is a useful adjunct to other measures of maturity.

The presence or absence of a hymen has been used in some studies to indicate maturity. However it should not be used for that purpose because adolescent (premature) mating occurs in at least some species of sharks, including blue sharks (Pratt, 1979). Furthermore, the absence of a hymen may not even be a good indicator of mating: we observed

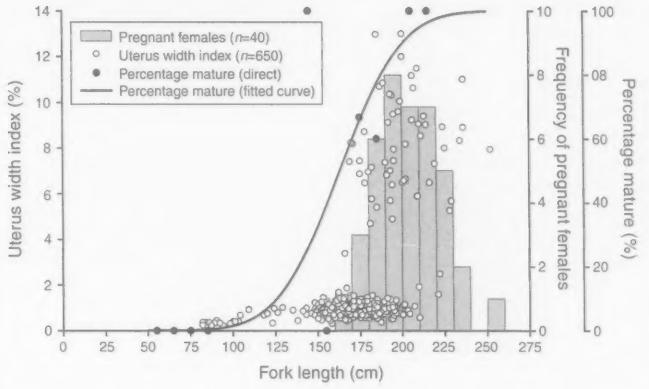

Figure 8

Maturation of female blue sharks (*Prionace glauca*): variation in uterus width index, direct maturity estimation from a suite of maturity indicators, and length-frequency distribution of pregnant females are shown.

Table 4

Summary of length at maturity indicators in porbeagle, shortfin mako, and blue sharks, and estimates of median length at maturity. Table entries are fork lengths in centimeters. Direct maturity estimates were derived by examination of a suite of maturity indicators. Italics indicate estimates based on small sample sizes over the maturation length range. "—" indicates that an estimate was not possible.

Sex	Maturity indicator	Porbeagle shark	Shortfin mako shark	Blue shark
Males	50% with spermatophores	152	178	194
	Rapid clasper elongation complete	143	185	—
	Direct maturity estimate	—	*183*	192
	Median length at maturity	140–150	180–185	190–195
Females	Rapid expansion of uterus begins	*145*	*275*	170
	First females pregnant	167	—[1]	166
	Direct maturity estimate	—	*280*	—
	Median length at maturity	170–180	275–285	170–190

[1] Only one pregnant female (290 cm FL) has been recorded from New Zealand.

some female shortfin makos in which the membrane was very thin and partially perforated, but had clearly not been damaged by copulation. We believe that the hymen disintegrates naturally with growth in makos; the same possibility was proposed for blue sharks by Pratt (1979).

Using a combination of our direct maturity estimates, and other indicators of maturity based on larger samples of sharks, we generated overall estimates of median maturity for both sexes of the three pelagic sharks (Table 4).

Porbeagle shark

In male porbeagles, the length at which 50% of sharks had spermatophores (152 cm) was longer than the length at which clasper elongation was complete (143 cm) (Table 4). However the percentage of males having spermatophores did not reach 100% in the longer length groups (Fig. 2), indicating that some mature males were reproductively inactive. This finding is consistent with reports from the western North Atlantic that male porbeagles have a seasonal cycle of spermatophore production, with a minimum in winter–spring (Jensen et al., 2002). If some mature males lacked spermatophores, the length at which 50% of males had spermatophores in our study was probably greater than the median maturity. The lack of a direct maturity estimate limits our ability to estimate the median maturity, but it is likely in the range 140–150 cm.

Similarly, we have no direct estimate of female porbeagle maturity. There was a considerable gap between

the length at which rapid expansion of the uterus began (145 cm) and the length of the smallest pregnant female (167 cm). UWI values less than 2% occurred for females up to about 185 cm (Fig. 3), but this does not mean that a high proportion of females in this length group had narrow uteri; uterus width measurements were not available for most of our pregnant females and therefore large UWI values are underrepresented in Figure 3. Most pregnant females were 170–200 cm long. We estimate that median maturity in females is about 170–180 cm, but this estimate requires confirmation. It is essentially the same as that provided by Francis and Stevens (2000) for New Zealand and Australian porbeagles (their New Zealand data were a smaller subset of the data used in the present study).

Although our estimates of median maturity for both males and females are uncertain, it is clear that porbeagles from New Zealand mature at considerably smaller lengths than they do in the North Atlantic. In the western North Atlantic, males mature at about 166 cm and females at 208 cm (Jensen et al., 2002). Data from the eastern North Atlantic (Gauld, 1989; Ellis and Shackley, 1995) are insufficient to estimate length at maturity, but the pregnant females reported by Gauld (1989) were considerably longer (199–248 cm) than those from New Zealand.

Porbeagles from the North Atlantic also grow larger than those from New Zealand: in the North Atlantic, sharks longer than 200 cm are common (Gauld, 1989; Campana et al., 2001), whereas around New Zealand and Australia they are very rare (Francis et al., 2001; Stevens and Wayte[4]). Differences in length at maturity between the North Atlantic and New Zealand and the proportion of sharks in the longer length classes indicate the existence of separate populations in the two regions—a conclusion that is supported by the disjunct distribution of porbeagles in the Northern and Southern Hemispheres (Compagno, 2001).

Shortfin mako shark

Our direct maturity estimate for male makos (183 cm) was based on a small sample size, and the small overlap between the lengths of immature and mature sharks is implausible. However, the lengths at which clasper development was completed, and at which 50% of males had spermatophores, were similar to the direct estimate (Table 4). Median maturity for males is therefore about 180–185 cm.

Our direct maturity estimate for female makos (280 cm) was based on few sharks over the maturation length range but was consistent with the length at which rapid uterus expansion began (275 cm). Our best estimate of median maturity in females is 275–285 cm.

Stevens (1983) used the degree of clasper calcification and an inflection in clasper length to estimate the length at maturity of males from New South Wales as 176 cm. In South Africa, males were estimated to mature at 177–188 cm (Cliff et al., 1990), but very few immature sharks were available. Our estimate of median maturity in New Zealand males (180–185 cm) is therefore similar to those from elsewhere.

Mollet et al. (2000) reported lengths at maturity for female makos of 298 cm total length in the Northern Hemisphere and 273 cm total length in the Southern Hemisphere. However, some of the 25 cm difference was due to Northern Hemisphere measurements having been taken over the curve of the body and Southern Hemisphere measurements having been taken in a straight line. Using appropriate conversion regressions, their Northern Hemisphere median maturity is equivalent to 267 cm FL, and their Southern Hemisphere median maturity is equivalent to 248 cm FL. When Mollet et al.'s Southern Hemisphere data are analysed separately for two subregions, South Africa and Australia, the estimated lengths at maturity are 244 cm (n=50) and 254 cm (n=32) respectively (Mollet[5]). The former is consistent with Cliff et al.'s (1990) estimate of 243 cm for South Africa, and the latter is consistent with Stevens's (1983) estimate of 255 cm for eastern Australia (both those estimates were made from subsets of the data used by Mollet et al. [2000]).

Our estimate of median maturity in New Zealand females (275–285 cm) is substantially higher than Mollet's[5] estimate for Australia (254 cm). Because tagged makos have moved between New Zealand and eastern Australia in both directions (Chan, 2001; Hartill and Davies, 2001; Holdsworth and Saul, 2003), we think it is unlikely that the difference is due to the presence of distinct populations in the two regions. We suspect that the difference is a result of possible length estimation errors (some of the Australian shark lengths were calculated from recorded weights, with a length-weight regression [Stevens, 1983; Mollet et al., 2000]), and the result of small sample sizes over the length range at maturation. For our direct maturity estimate, we had only 19 New Zealand sharks over the length range 240–290 cm, and Mollet[5] had 15 sharks.

Interestingly, our estimate of median maturity in New Zealand females is also greater than Mollet et al.'s (2000) estimate for the western North Atlantic, thus removing the reported between-hemisphere difference. We believe that larger, accurately measured samples of female makos are required before definitive statements can be made about length at maturity in the various regions.

Blue shark

In male blue sharks from New Zealand, CLI lacked an inflection near the length of maturity—a feature that

[4] Stevens, J. D., and S. E. Wayte. 1999. A review of Australia's pelagic shark resources. FRDC Proj. Rep. 98/107, 64 p. [Available from CSIRO Marine Research, PO Box 1538, Hobart, Tasmania 7001, Australia.]

[5] Mollet, H. 2004. Personal commun. Moss Landing Marine Laboratories, 8272 Moss Landing Road, Moss Landing, CA 95039.

has also been reported elsewhere (Pratt, 1979; Hazin et al., 1994). Thus clasper length was not useful in estimating length at maturity. Our direct maturity estimate was similar to the length at which 50% of sharks had spermatozeugmata and indicated that median maturity occurs at about 190–195 cm (Table 4).

In females, maturation occurred over a wide length range, as reported elsewhere (Hazin et al., 1994). Taking into account the length distributions of pregnant females and females with low UWI values (Fig. 8), we believe median maturity is likely in the range 170–190 cm.

In other blue shark studies, estimation of the length at maturity has also been hindered by small sample sizes, or even a complete absence of immature or mature sharks. In the western North Atlantic, males mature at about 178 cm, and females at around the same length, although few mature females have been available (Pratt, 1979). In the Gulf of Guinea, Atlantic Ocean, 50% of females were pregnant at 180 cm (Castro and Mejuto, 1995). In Australian studies, a lack of immature sharks made it impossible to estimate maturity adequately (Stevens, 1984; Stevens and McLoughlin, 1991). In the North Pacific Ocean, 50% of males had spermatozeugmata at 166 cm and 50% of females were pregnant at 174 cm (Nakano, 1994). Thus worldwide estimates of maturity in blue sharks are similar to ours from New Zealand, except perhaps for a smaller length at maturity of males in the North Pacific. Unlike females in most species of sharks, female blue sharks do not appear to mature at a length greater than that for mature males.

Acknowledgments

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Survey- and fishery-derived estimates of Pacific cod (*Gadus macrocephalus*) biomass: implications for strategies to reduce interactions between groundfish fisheries and Steller sea lions (*Eumetopias jubatus*)

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Abstract—Survey- and fishery-derived biomass estimates have indicated that the harvest indices for Pacific cod (*Gadus macrocephalus*) within a portion of Steller sea lion (*Eumetopias jubatus*) critical habitat in February and March 2001 were five to 16 times greater than the annual rate for the entire Bering Sea-Aleutian Islands stock. A bottom trawl survey yielded a cod biomass estimate of 49,032 metric tons (t) for the entire area surveyed, of which less than half (23,329 t) was located within the area used primarily by the commercial fishery, which caught 11,631 t of Pacific cod. Leslie depletion analyses of fishery data yielded biomass estimates of approximately 14,500 t (95% confidence intervals of approximately 9,000–25,000 t), which are within the 95% confidence interval on the fished area survey estimate (12,846–33,812 t). These data indicate that Leslie analyses may be useful in estimating local fish biomass and harvest indices for certain marine fisheries that are well constrained spatially and relatively short in duration (weeks). In addition, fishery effects on prey availability within the time and space scales relevant to foraging sea lions may be much greater than the effects indicated by annual harvest rates estimated from stock assessments averaged across the range of the target species.

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Fish. Bull. 103:501–515 (2005).

For the past 30 years, the Steller sea lion (*Eumetopias jubatus*) population in western Alaska has declined (Braham et al., 1980; Sease and Gudmundson[1]). The species was listed as threatened under the U.S. Endangered Species Act (ESA) in 1990 after evidence of a major decline in abundance in the core of its range from the Kenai Peninsula in south-central Alaska to Kiska Island in the western Aleutian Islands (Braham et al., 1980; Merrick et al., 1987). After the decline was first observed in the eastern Aleutian Islands in the early 1970s (Braham et al., 1980), it spread eastward to Prince William Sound and westward through Russia during the next decade (Merrick et al., 1987; Loughlin et al., 1992). From the early 1970s to 1990, counts of adult and juvenile Steller sea lions declined by over 70%, but annual rates of decline were most severe between 1985 and 1989 (–15%/yr; Loughlin et al., 1992). During the 1990s, the decline slowed to approximately –5%/yr and may have temporarily abated in many areas by 2002 (Sease and Gudmundson[1]).

Understanding the causes for the decline and lack of recovery in the Steller sea lion population has largely eluded scientists and managers, despite the millions of dollars spent on scientific research (Ferrero and Fritz[2]) and numerous reviews by academic (Alaska Sea Grant[3]; DeMaster and Atkinson[4]; NRC, 1996; 2003) and governmental panels (Kruse et al.[5]; NMFS[6,7,8,9]). Although recent reviews

[1] Sease, J. L., and C. J. Gudmundson. 2002. Aerial and land-based surveys of Steller sea lions (*Eumetopias jubatus*) from the western stock in Alaska, June and July 2001 and 2002. NOAA Tech. Memo. NMFS-AFSC-131, 45 p. Alaska Fisheries Science Center, 7600 Sand Point Way NE, Seattle WA 98115.

[2] Ferrero, R. C., and L. W. Fritz. 2002. Steller sea lion research coordination: a brief history and summary of recent progress. NOAA Tech. Memo. NMFS-AFSC-129, 34 p. Alaska Fisheries Science Center, 7600 Sand Point Way NE, Seattle WA 98115.

[3] Alaska Sea Grant. 1993. Is it food?: Addressing marine mammal and seabird declines. Workshop summary rep. AK-SG-93-01, 59 p. Univ. Alaska Fairbanks, Alaska Sea Grant College Program, Fairbanks AK 99775.

[4] DeMaster, D., and S. Atkinson. (eds.). 2002. Steller sea lion decline: Is it food? II. Workshop summary. rep. AK-SG-02-02, 80 p. Univ. Alaska Fairbanks, Alaska Sea Grant College Program, Fairbanks AK 99775.

[5, 6, 7, 8, 9] *See next page.*

(Kruse et al.[5]; DeMaster and Atkinson[4]; NRC, 2003) concluded that "top-down" forces, such as predation or illegal shooting, are greater threats to recovery of the Steller sea lion population, they could not eliminate "bottom-up" factors from consideration. NRC (2003) suggested that NMFS conduct an adaptive management experiment to determine the magnitude of one such "bottom-up" force, nutritional stress resulting from competition with fisheries for prey (NMFS[6,7,8,9]; NRC, 2003). The North Pacific is home to some of the largest fisheries in the world, particularly those for groundfish such as Pacific cod (*Gadus macrocephalus*) and walleye pollock (*Theragra chalcogramma*). Steller sea lions eat a wide variety of fish and cephalopods, including Pacific cod, walleye pollock, Atka mackerel (*Pleurogrammus monopterygius*), arrowtooth flounder (*Atherestes stomias*), salmon (*Oncorhynchus* spp.), herring (*Clupea pallasi*), capelin (*Mallotus villosus*), eulachon (*Thaleichthys pacificus*), sand lance (*Ammodytes hexapterus*), squid, and octopus (Sinclair and Zeppelin, 2002). A large proportion of their diet, however, is composed of semidemersal or pelagic schooling fish, particularly fish in spawning migrations or aggregations nearshore. These same species are often targeted at the same time and in the same areas by groundfish fisheries, particularly those fisheries that use trawl gear. Concerns about the potential of fisheries to create localized depletions of prey in important sea lion foraging habitats have led to controversial groundfish fishery restrictions throughout most of Alaska (NMFS[8,9]).

[5] Kruse, G. H., M. Crow, E. E. Krygier, D. S. Lloyd, K. W. Pitcher, L. D. Rea, M. Ridgway, R. J. Small, J. Stinson and K. M. Wynne. 2001. A review of proposed fishery management actions and the decline of Steller sea lions (*Eumetopias jubatus*) in Alaska: a report by the Alaska Steller sea lion restoration team. Regional information report 5J01-04, 106 p. Alaska Dep. Fish and Game, P.O. Box 25526, Juneau AK 99802.

[6] NMFS (National Marine Fisheries Service). 1998. Endangered Species Act Section 7 Consultation on an Atka mackerel fishery under the BSAI groundfish FMP between 1999 and 2002; authorization of a walleye pollock fishery under the BSAI FMP between 1999 and 2002; and under the GOA FMP between 1999 and 2002, 189 p. NMFS Protected Resources Division, Alaska Region, P.O. Box 21668, Juneau, AK 99802.

[7] NMFS. 2000. Endangered Species Act. Section 7: Consultation, biological opinion and incidental take statement on the authorization of the Bering Sea-Aleutian Islands and Gulf of Alaska groundfish fisheries based on the Fishery Management Plans, 352 p. NMFS Protected Resources Division, Alaska Region, P.O. Box 21668, Juneau, AK 99802.

[8] NMFS. 2001. Endangered Species Act. Section 7: Consultation, biological opinion and incidental take statement on the authorization of the Bering Sea-Aleutian Islands and Gulf of Alaska groundfish fisheries based on the Fishery Management Plans as modified by Amendments 61 and 70, 206 p. NMFS Protected Resources Division, Alaska Region, P.O. Box 21668, Juneau, AK 99802.

[9] NMFS. 2003. Supplement to the Endangered Species Act. Section 7: Consultation, biological opinion and incidental take statement of October 2001, 179 p. NMFS Protected Resources Division, Alaska Region, P.O. Box 21668, Juneau, AK 99802.

Assessment models and fisheries harvest strategies have determined the overall fishing mortality rate that can be allowed for the stock and the amount of biomass that can be removed. In practice, however, catches are not uniformly distributed across the range of the assessed stock nor are they distributed equally throughout the year. Although there is evidence that the Atka mackerel trawl fishery has created localized depletions of its target species (NMFS[6] Lowe and Fritz, 1997; NRC, 2003), this finding has not been generally applied to fisheries for other sea lion prey. Trawl fisheries in the Aleutian Islands may have, in certain instances, reduced local abundances of Atka mackerel by as much as 90% (Lowe and Fritz, 1997). Atka mackerel and its fishery have characteristics that permitted analysis of fishery data in this way. The species does not possess a swim bladder and thus makes a poor acoustic target. As a consequence, the Atka mackerel fishery does not target on an acoustic signal, but instead trawls in areas where the species is known to congregate. Through the analysis of time series of catch and effort statistics from local fisheries with Leslie's equation (Ricker, 1975; Hilborn and Walters, 1992; Gunderson, 1993), estimates of the initial abundance of Atka mackerel (prefishery) and its catchability (proportion of the stock caught with one unit of effort) were made within the context of certain assumptions, which included the following: 1) the population being fished is closed, or alternatively that immigration and growth are equal to emigration plus natural mortality, 2) catchability over the course of the fishery remains constant, and 3) changes in catch per unit of effort (CPUE) are directly related to changes in fish density. These assumptions may be met for marine species if the area fished is well defined (e.g., is surrounded by habitat that is unsuitable for the species), the duration of the fishing season is relatively short, or the species is relatively sedentary (Polovina, 1986; Ralston, 1986; Joll and Penn, 1990; Miller and Mohn, 1993). Although they indicate that fisheries have created local depletions of Atka mackerel, these models are difficult to apply to other North Pacific fisheries because of a lack of fishery-independent estimates of biomass and by circumstances unique to the Atka mackerel fishery (e.g., the fishery trawls in areas where the species is known to congregate rather than uses acoustic signal, Atka mackerel are patchily distributed, and patches are separated by areas with low fish density).

To obtain information on the winter distribution of groundfish in areas used by foraging Steller sea lions and groundfish fisheries, the Alaska Fisheries Science Center of the National Marine Fisheries Service conducted a bottom trawl survey for groundfish in the southeastern Bering Sea north of Unimak Island in February–March 2001 (Fig. 1). This area is important to the Pacific cod fishery in winter because cod aggregate in this area to spawn (Shimada and Kimura, 1994). It is also recognized as an important foraging area for Steller sea lions because it is designated as critical habitat under the ESA (NMFS[7,8]).

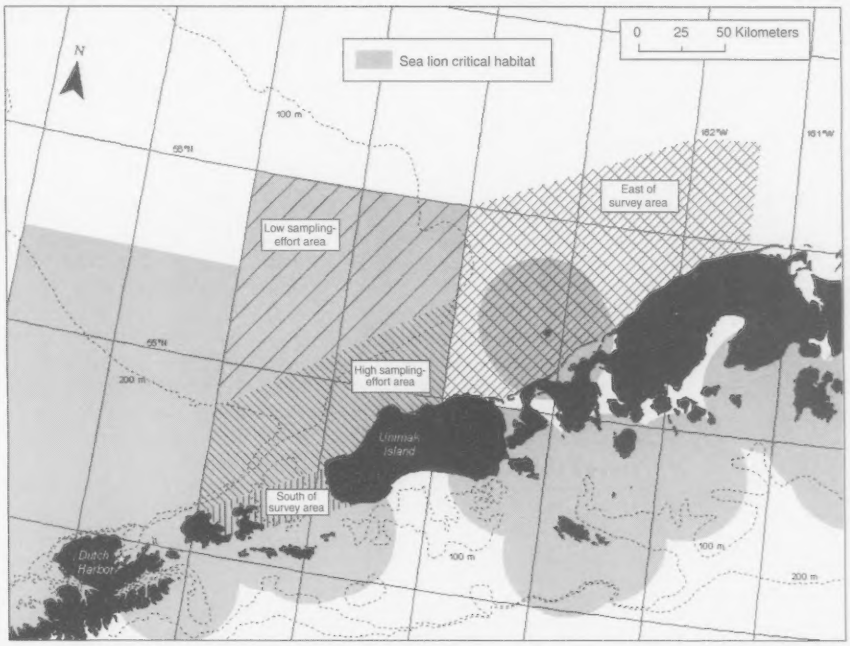

Figure 1

The four areas (high and low sampling-effort survey areas, the area east of the survey area, and the area south of the survey area) in the southeastern Bering Sea that were surveyed in February–March 2001 for groundfish with a bottom trawl and used for analysis of Pacific cod (*Gadus macrocephalus*) fishery data. Steller sea lion (*Eumetopias jubatus*) critical habitat is also shown.

In this article, estimates of Pacific cod biomass from Leslie depletion analyses of fishery data are compared with those derived from a bottom trawl survey conducted in the same area at the same time. These two methods are independent because they use completely different data to estimate the same parameter, Pacific cod biomass. If they yield similar results, they would support each other in the estimate of local area cod biomass and support the use of Leslie depletion analyses of data from relatively short and spatially well-defined fisheries operations for making such estimates. Furthermore, these comparisons increase our understanding of the potential local effects of a fishery in areas important for sea lion foraging and permit comparison with the results of assessments of the Pacific cod stock in the entire eastern Bering Sea (Thompson and Dorn, 2002). In this instance, if the change in Pacific cod abundance attributable to the fisheries north of Unimak Island is not greater than what would have occurred if catch were evenly distributed throughout the year and across the range of the stock, then it could be argued that no localized depletion occurred. However, if the local change in abundance is greater than expected, does this constitute a localized depletion of the species? The answer ultimately depends on the extent to which the fishery negatively affects the target species (e.g., by reducing recruitment) or, as in our case, by reducing the foraging success of sea lions, which, in turn, could lead to reduced survival or reproductive rates. Although we do not know what the threshold levels of change in local prey densities are for foraging Steller sea lions, it is first necessary to determine the level of change in local abundance that may be attributable to fisheries.

There are several aspects of Pacific cod life history in the eastern Bering Sea that make it difficult to use fishery data and the Leslie depletion method to estimate local area biomass. The most important may be that the population in the area fished may not be closed over the time period analyzed. Pacific cod spawn north of Unimak Island in late winter but apparently arrive in groups and, after spawning, leave the area and spread out on the eastern Bering Sea shelf to feed during the remainder of the year (Shimada and Kimura, 1994; Thompson and Dorn, 2002). Seasonal emigration from and immigration into spawning areas in critical habitat, modeled with a combination of fishery and survey data by NMFS scientists[10] (Fig. 2), provide a baseline

[10] NMFS. 2000. Estimation of monthly Pacific cod biomass inside Steller sea lion critical habitat. *In* Biological opinion questions, NMFS-AKC analytical team. Unpubl. manuscript, 112 p. Alaska Fisheries Science Center, 7600 Sand Point Way NE, Seattle WA 98115.

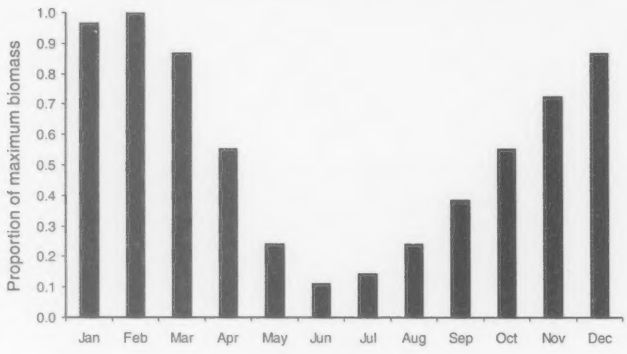

Figure 2

Proportion of maximum (in February) biomass of Pacific cod (*Gadus macrocephalus*) within Steller sea lion (*Eumetopias jubatus*) critical habitat in the eastern Bering Sea by month (see Footnote 10 in the general text).

against which possible changes related to local fisheries can be compared. The model results indicate that the highest biomass in critical habitat (largely on the shelf north of Unimak Island) occurs in February, declines to about 10% of the peak in June, and then slowly rebuilds through the summer and fall. Changes in the behavior of Pacific cod immediately prior to or after spawning, such as the formation of dense aggregations or the temporary cessation of feeding, would affect catchability by both trawl and fixed gears. However, abrupt changes in catchability due to the formation of aggregations should be evident within the time series of catch and effort data, and changes in feeding habits would not affect the catchability by trawl gear.

Methods

Bottom trawl survey

Stations sampled during the bottom trawl survey were selected by using a stratified random scheme. Two strata were defined: one with a high and another with a low degree of sampling effort, based on the expected distribution and abundance of Pacific cod from fishery information. In the nearshore or high sampling-effort stratum (7765 km^2), 38 stations were sampled, whereas 19 stations were sampled in the larger (12,112 km^2), offshore low sampling-effort stratum (Fig. 1). All survey tows were conducted during daylight hours from 16 February to 1 March 2001 aboard the FV *Northwest Explorer* and the FV *Ocean Harvester*. The 49-m FV *Northwest Explorer* was equipped with two 1800-hp engines, and the 33-m FV *Ocean Harvester* had a single 1250-hp engine. Both vessels were house-forward trawlers that had stern ramps, multiple net storage reels, and paired hydraulic trawl winches with 1280–2190 m of 2.54-cm diameter steel cable. Each vessel carried a full complement of navigation and fishing electronics, including global positioning systems (GPS), video position plotters, radars, and depth sounders.

A Poly-Nor'eastern high-opening bottom trawl rigged with roller gear was used to sample the groundfish community at each selected location. The trawl net was constructed of 12.7-cm stretched-mesh polyethylene web and had a 3.2-cm stretched-mesh nylon liner in the codend. Accessory gear for the Nor'eastern trawl included three 54.9 m, 1.6 cm diameter galvanized wire rope bridles, and 1.8 × 2.7 m steel V-doors weighing approximately 850 kg each.

Biomass (B) estimates for each stratum surveyed were computed by multiplying the average CPUE (in units of kg/km^2) for all hauls (n) in a stratum by its area (A). Haul CPUE was calculated as the weight of cod caught (kg) divided by the area swept (a), which was the length of the tow multiplied by the average net width determined by sonic mensuration equipment:

$$B = \frac{\sum^{n} \frac{kg}{a}}{n} \times A.$$

Confidence bounds on stratum biomass estimates were computed from the standard deviation of the haul CPUEs. For haul CPUEs we assumed a catchability[11] of 1 for Pacific cod (all cod within the area swept by

[11] Note that catchability within the survey biomass estimation procedure has a different literal definition than in the Leslie equation.

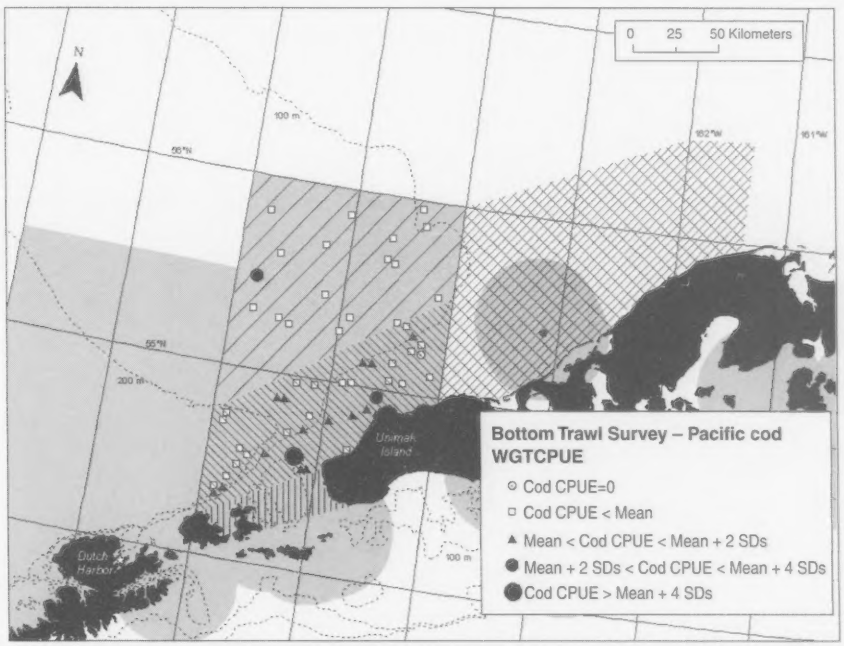

Figure 3

Catch per unit of effort (CPUE=kg/km^2) of Pacific cod (*Gadus macrocephalus*) during the February–March 2001 bottom trawl survey of the southeastern Bering Sea. "Wgtcpue" refers to the CPUE of Pacific cod from individual hauls (Table 2). Area shading is the same as that in Figure 1.

the net are captured) and that it is constant over the course of the survey. This assumption is also made in the Leslie analyses of fishery data. In addition, each haul is assumed to be a random, normally distributed estimate of the density of cod within the stratum. Therefore, the average of the haul CPUEs of cod was assumed to be an unbiased estimate of the true density of cod, allowing linear extrapolation from the CPUE within the area swept to a biomass estimate for each stratum.

Analysis of fishery data

Fishery observers record a wide variety of information about each haul taken by a fishing vessel, including retrieval location, depth, date and time of catch, and total catch weight (all referred to hereafter as "haul data"). In addition, the catch of a randomly chosen subset of hauls was sampled to determine the species composition of the haul and the length distribution of the target species (see Nelson et al. 1981 and NMFS[12] for observer sampling methods). Observer data were queried for any hauls with any gear in which Pacific cod were caught in the eastern Bering Sea and Aleutian Islands region in 2001. The geographic distribution of the observed Pacific cod catch was used to estimate the distribution of the actual catch of Pacific cod from January–April 2001 in four areas of the southeastern Bering Sea (Fig. 1): the high and low sampling-effort areas surveyed in February–March 2001, and two areas outside of the area surveyed—one to the east, and one to the south. To account for Pacific cod catches in both unsampled hauls and on unobserved vessels, the observed catch of cod was multiplied by the ratio of total-to-observed catch by processing sector and gear type (Table 1). For this procedure, the catch of the unobserved portion of the fleet is assumed to be similar to the observed portion. Ratios of total-to-observed catch by sector and gear ranged from 1.02 to 33.94, but for the majority of the catch, the ratios were less than 2 (Table 1).

A simple Leslie analysis of fishery catch and effort data was conducted on data collected by observers onboard vessels targeting groundfish. For the basic Leslie model (Ricker, 1975; Hilborn and Walters, 1992; Gunderson, 1993) a deterministic linear relationship between CPUE and cumulative catch is assumed:

$$\frac{C_t}{f_t} = qB_0 - qK_t,$$

[12] NMFS. 1996. Manual for biologists aboard domestic groundfish vessels, 431 p. U.S. Dep. Commer., NOAA, NMFS, Alaska Fisheries Science Center, 7600 Sand Point Way, NE, Seattle, WA 98115.

Table 1

Observed and total estimates of total catches of Pacific cod by processor and gear type in the Bering Sea-Aleutians Island region in 2001, and the ratio of Total ÷ Observed catches. CP=catcher processor; CV=catcher vessel.

Gear	Catches and ratio	Processor type		
		CP	CV	Other
Trawl	Total (t)	29,398	21,354	734
	Observed (t)	19,316	8590	720
	Ratio	1.52	2.49	1.02
Hook and line	Total (t)	96,238	637	11,331
	Observed (t)	52,920	19	11,109
	Ratio	1.82	33.94	1.12
Pot	Total (t)		16,506	478
	Observed (t)		4741	469
	Ratio		3.48	1.02

where C_t = catch in time period t;
f_t = effort in t;
q = catchability;[11]
B_0 = underlying (or initial) biomass; and
K_t = cumulative catch through t.

Current catch, effort, and cumulative catch are required by the model, whereas catchability and initial biomass are estimated from it. The catch and effort time series used in these analyses were 1) daily aggregates of observed cod catch in metric tons (t) and effort by vessels targeting cod by area (i.e., the high sampling-effort [HSE] area, the low sampling-effort area [LSE], the area east [AE] and the area south [AS] of the survey area), and 2) daily cumulative catch of cod by area for all vessels. CPUE metrics were defined for each gear: 1) trawl as the catch of cod (t) per hour of observed trawling per day; 2) pot as the catch of cod (t) per 20 pots observed per day; and 3) hook and line as the catch of cod (t) per 1000 hooks observed per day. These metrics were chosen so that the CPUE for each gear would be in approximately the same range to permit being plotted together on the same axis. Changing the unit-of-effort definition (number of pots or hooks fished, for instance) has no effect on the significance of the results. Hauls for which cod was the target species were defined as those in which the catch of cod was at least 20% of the total groundfish catch; target levels of 40% and 60% were also explored for trawl fisheries. Catch and effort from these hauls alone, in which cod was the target species, were used for CPUE calculations, whereas cumulative catch was derived from the total catch of cod from all vessels regardless of their target species.

The relationship between trawl vessel length and CPUE was investigated but was not included in the Leslie analyses. It was expected that CPUE would be directly related to vessel length. With increasing vessel length, horsepower would increase as would the vessel's ability to use larger nets. Vessel length (a surrogate variable for horsepower) could be a significant covariate in the relationship between CPUE and cumulative catch.

Results

Bottom trawl survey

Mean CPUE (kg/km^2) of Pacific cod in the smaller HSE survey stratum was almost three times higher than in the larger LSE stratum, resulting in mean biomass estimates of 31,312 t and 17,720 t of Pacific cod, respectively (Table 2 and Fig. 3). The highest recorded CPUE of cod was recorded for a haul on the northeast side of Unimak Pass (Fig. 3). Hauls with CPUEs above the mean were distributed throughout the HSE stratum in depths less than 200 m. Only one of the 18 hauls in the LSE stratum had a CPUE larger than the mean. For the HSE stratum, the 95% confidence interval on the mean biomass estimate was 19,284–43,339 t.

Fishery data

Total catch of Pacific cod Approximately 30,500 t of Pacific cod were caught in the four areas of the southeastern Bering Sea from 1 January to 30 April 2001 (Table 3 and Fig. 4). Almost 60% of this total catch was collected in the HSE survey stratum, whereas 25% and 12% of the total catch were collected in the AE and AS of the survey area, respectively; only 4% was collected in the LSE survey stratum. Based on the distribution of the observed catch of cod by gear, approximately half of the total catch was collected by trawls, a third by hook and line (=longline), and 14% by pots.

The distribution of cod catch by area primarily reflects the distribution of the fishery targeting Pacific cod (Fig. 4). Of the 5813 t of cod that was observed caught by the cod trawl fleet (with at least 20% of each haul composed of cod), 86% was caught in the HSE stratum in over 4600 hours of observed trawling. Most of the remainder (13% or 781 t) was caught east (AE) of the survey area, primarily between the HSE stratum and the 20 nautical mile (nmi) radius trawl exclusion zone encompassing sea lion critical habitat around Sea Lion Rocks and Amak Island (Figs. 1 and 4). There was little trawl effort targeting Pacific cod in the LSE stratum (only 10 observed hours of trawling) or south (17 hours observed) of the survey area. The cod pot fleet worked primarily south of the survey area (57% of their catch) and in the HSE stratum (31%) in areas where conflicts with trawl gear would be minimized. The cod longline fleet worked in both the HSE stratum and to the east of the survey area, and had only trace amounts of catch in the other areas (Table 3).

Percentage of Pacific cod in the haul The distribution of the percentage of cod in the total catch of each haul

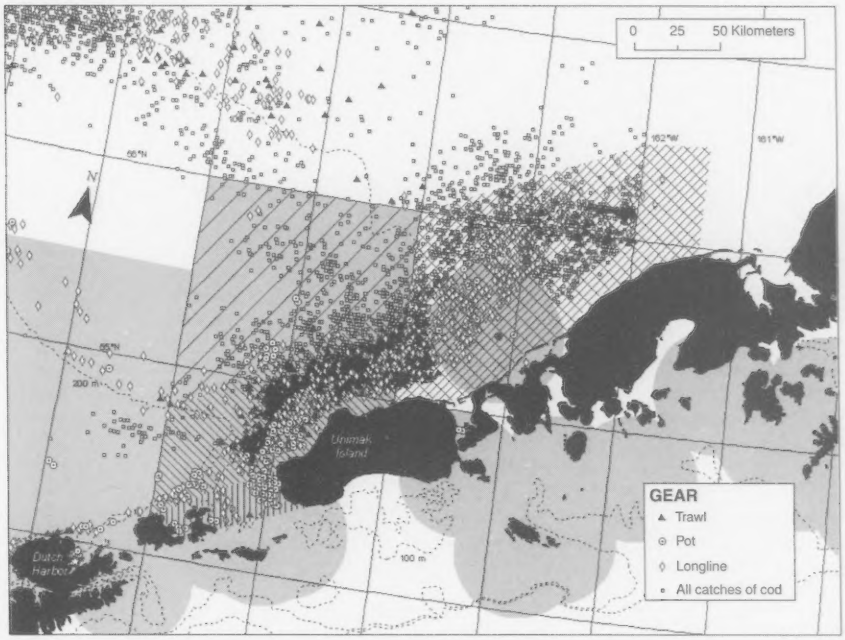

Figure 4

Locations of groundfish fishery catches of Pacific cod (*Gadus macrocephalus*) in the southeastern Bering Sea, January–April 2001. The cod target fishery is separated by gear type (trawl=at least 20% of the haul by weight was cod). "All catches of cod" refers to bycatch in trawl fisheries targeting other species. Area shading is the same as that seen in Figure 1.

Table 2

Results (catch and biomass of Pacific cod) and haul data from the bottom trawl survey of the southeastern Bering Sea conducted in February–March 2001. Low and high sampling-effort strata are shown in Figure 1. (CPUE=catch per unit of effort; CI=confidence interval).

	Survey stratum		
	Low sampling effort	High sampling effort	Total
Number of hauls	19	38	57
Number of hauls with cod	19	37	56
Mean CPUE (kg cod/km^2)	1463	4032	3176
Range in CPUE	65–12,681	0–21,299	0–21,299
Standard deviation of CPUE	2776	4676	4292
Area of stratum (km^2)	12,112	7765	19,877
Area of stratum sampled (km^2)	0.472	0.927	1.399
% of stratum area sampled	0.004%	0.012%	0.007%
Biomass (t)	17,720	31,312	49,032
95% CI on biomass (t)	1513–33,928	19,284–43,339	20,796–77,267

indicates that the vast majority of the fleet using pots or longline gear were targeting Pacific cod. The total catch of 350 of 351 observed hauls of pots and 777 of 797 observed hauls of longlines was composed of at least 60% cod (Table 4). Therefore, use of a 20% threshold to identify the cod fleet for the longline and pot vessels was unnecessary. For the trawl fleet, however, more than half the observed hauls had less than 10% cod, and

63% had less than 20% cod. These trawl vessels were targeting fish species other than Pacific cod, such as rock sole, and caught some cod (as bycatch) in the process. The distribution of hauls that had greater than 20% cod (by 10% bins) was relatively flat, varying only from 4% to 7% between bins and having no clear threshold or breakpoint. Use of a low threshold proportion of cod (such as 20%) would likely include some hauls in which

Table 3

Catch and effort statistics for Pacific cod fisheries in the southeastern Bering sea by strata (Fig. 1) in January–April 2001. Statistics include total catch estimates (in metric tons (t); all gear and fisheries), observed catch by all fisheries (by gear type), and observed catch and effort by fisheries targeting Pacific cod (by gear type). Three levels of Pacific cod catches from trawl gear are listed and are based on the minimum proportion of cod in each haul.

	Strata				
	East of sampling area	High sampling effort	Low sampling effort	South of sampling area	Total
Catch					
Total catch	7691	17,875	1,200	3724	30,491
Observed catch—all fisheries					
Trawl	1628	5737	324	32	7720
Pot	85	655	152	1198	2091
Longline	2493	2001	45	116	4654
Total	4205	8393	521	1345	14,465
Observed catch—Pacific cod fisheries					
Trawl (20% cod in each haul)	781	4993	7	32	5813
Trawl (40% cod in each haul)		4119			
Trawl (60% cod in each haul)		3364			
Pot	85	655	152	1198	2090
Longline	2493	2001	45	116	4654
Effort					
Trawl (hours; 20% cod in each haul)	677	4644	10	17	5348
Trawl (hours; 40% cod in each haul)		3768			
Trawl (hours; 60% cod in each haul)		2903			
Pot (number of pots)	1857	10,130	1119	14,816	27,922
Longline (no. of hooks)	4,220,051	3,265,606	88,880	165,585	7,740,122

Table 4

Frequency distribution of the percentage of cod in each haul by gear for the groundfish fishery in the four areas of the eastern Bering Sea (Fig. 1) in January–April 2001

	Trawl		Longline		Pot	
% cod	No. of hauls	% of total	No. of hauls	% of total	No. of hauls	% of total
<10%	1810	52	0	0	0	0
10–20%	371	11	1	0	0	0
20–30%	237	7	2	0	1	0
30–40%	169	5	1	0	0	0
40–50%	126	4	5	1	0	0
50–60%	126	4	11	1	0	0
60–70%	151	4	40	5	2	1
70–80%	166	5	120	15	4	1
80–90%	181	5	334	42	37	11
90–100%	161	5	283	36	307	87
Total	3498		797		351	

other species were targeted. On the other hand, the use of a high threshold (such as 60%) might exclude hauls where Pacific cod was the target species. Therefore, a range of trawl target definitions from 20% to 60% was used. The cod trawl fleet distribution shown in Figure 4 was defined by the 20% threshold. If the 40% or 60% thresholds are used, most of the cod trawl effort shown in the HSE area remains, whereas some of the effort in the eastern portions of the AE of the survey area is not coded as the effort of a cod-target fishery.

Distribution of Pacific cod catch Cod catches accumulated differently in the three primary areas fished (Fig. 5). In the HSE area, cod catches rose steadily from 1 January through early April, and totaled approximately 13,000 t. There was a brief increase in the rate of cod catch in mid-April, but by approximately 20 April, the cod fishery in the HSE area had essentially finished with a catch total of 17,875 t. In the AE of the survey area, cod catches accumulated steadily from 1 January through 2 March, and totaled 6340 t. There was a brief increase in catch rates for 6 days from 25 through 30 March, after which the cod fishery in the AE of the survey area was finished with a catch total of 7691 t. In the AS of the survey area, there was little cod fishing effort prior to 22 February, and it lasted only through 27 March, by which time almost 3500 t had been caught; catches through 30 April from the AS of the survey area totaled 3724 t. There was very little cod fishery effort in the LSE area (Table 3), and only 1200 t of cod were caught (principally as bycatch in other fisheries) through 30 April 2001.

The longline fleet began fishing for Pacific cod in both the HSE area and AE of the survey area on 1 January (Fig. 5). In the HSE area, daily average longline CPUE (t cod per 1000 hooks per day) remained relatively low and steady, ranging from 0.3–0.7 through January. The longline fleet left the HSE area for approximately two weeks, resuming effort again on 13 February and continuing through 6 March. Longline CPUEs were generally higher in late February than they were in January, ranging from approximately 0.7 to 1.2. The longline fleet again returned to the HSE area on 19–24 March, but daily average CPUEs were <0.5. There was sporadic longline fishing for cod in the HSE area through April, and CPUEs ranged from 0.3 to 1.0. In the AE of the survey area, the longline fleet fished continuously from 1 January through 2 March, and daily average CPUE declined from a range of 0.7–1.0 on 1–7 January to a range of 0.3–0.5 on 24 February–2 March.

The trawl fishery for cod began on 20 January in both the HSE area and AE of the survey area (Fig. 5). In the HSE area, trawl CPUE (t cod per hour trawled per day) increased from a range of 0.7–1.4 on 20–27 January to a range of 1.3–2.5 on 6–15 February. From 16 February–1 March, trawl CPUEs were slightly lower, ranging from 0.8 to 2.0, after which they declined further, ranging only from 0.5 to 1.3 from 2–24 March. On 26 March, the average CPUE increased substantially to over 12 but quickly declined to less than 1.0 by 1 April. This was followed by another short-lived increase in CPUE on 11 April, after which daily average CPUEs remained below 1.0 through April. In the AE of the survey area, CPUEs were highly variable (between 0.4 and 2.3) and there was little observable trend between 20 January and early March. On 25 March, however, average CPUE increased to over 4 and ranged between 0.4 and 3.9 through 2 April, after which there was only sporadic effort and daily average CPUEs were less than 1.

The pot fishery for cod began on 22 February south of the survey area and on 24 February in the HSE area (Fig. 5). In the AS of the survey, pot CPUE (t cod per 20 pots per day) decreased from a range of 0.3–1.0 from 22 February–1 March, to a range of 0.2–0.5 on 8–17 March. However, on 18 March, pot CPUE increased to 1.1, and remained between 0.5 and 0.8 through 22 March, after which it quickly declined to very low levels. In the HSE area, pot CPUE ranged between 0.7 and 1.7 from 24 February to 23 March. However, on 24–25 March, CPUE was greater than 2. Pot cod fishing occurred on only three more days through the end of April in the HSE area: on 27 March, 6 April, and 12 April. Although daily average CPUEs on the last two days were the highest recorded in the pot fishery in 2001, observed catches on these days totaled only 4 and 5 t of cod, respectively.

Leslie depletion analyses Leslie depletion analyses were conducted on four sets of Pacific cod fishery data collected in the HSE area and on two sets of data collected in the AE of the survey area (Table 5). In the HSE area, longline fishery data collected prior to 13 February and trawl fishery data collected prior to 6 February were excluded from the analyses because CPUE data indicated that fish were immigrating into the area in January in preparation for spawning (Fig. 5). It is unlikely that the increase in CPUE was due to a change in catchability because the increase was evident whether bait was used (pots and longlines) or not (trawls). Data indicating an increase in the abundance of cod north of Unimak Island in January and a peak in February were in agreement with a generalized model of cod abundance in Steller sea lion critical habitat in the eastern Bering Sea (Fig. 2) and seasonal cod movements from tagging data (Shimada and Kimura, 1994). The time series was truncated at 24 March because of the evidence within the fisheries data (increase in CPUE) that another group of cod had immigrated to the HSE area and AE of the survey area in late March or that catchability had increased substantially (Fig. 5). In addition, daily average CPUEs from hauls that had at least 20%, 40%, and 60% Pacific cod by weight were regressed against cumulative catch to see what effect the target definition might have on the regression results.

All Leslie regressions with longline or trawl fishery data from the HSE area were highly significant ($P<0.000001$; Table 5 and Fig. 6). Coefficients of determination (r^2) for the longline and the trawl-20% data were both greater than 0.6. Regression coefficients

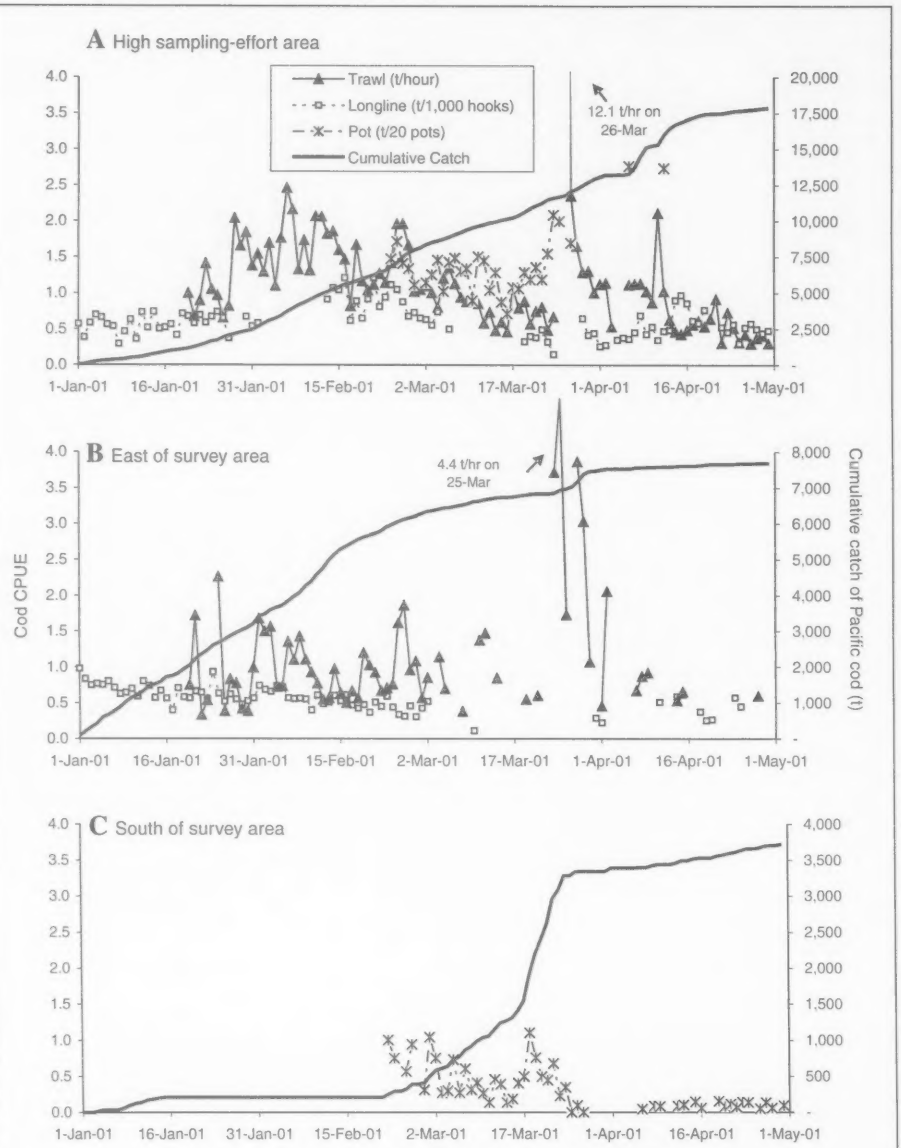

Figure 5

Daily average catch per unit effort (CPUE on left *y*-axis) for the observed Pacific cod (*Gadus macrocephalus*) fishery by gear (see legend for units) and area (Fig. 1) from 1 January–30 April 2001 in the southeastern Bering Sea. Estimated cumulative catch (t) of cod by all gear types by area is also shown (right *y*-axis).

(slopes) in all cases were negative and significantly different from zero. Collectively, these results strongly indicate that cod fishery CPUE was negatively correlated with cumulative catch. Initial biomass estimates (B_0) from the four regressions were similar and ranged between 14,119 and 14,806 t, with 95% confidence intervals ranging from approximately 9000 to 25,000 t. Use of different fishery catch levels (20%, 40%, 60% cod in each haul) had little effect on the initial biomass estimate but changed the estimate of q, which increased directly with the threshold proportion of cod in each haul (Table 5 and Fig. 7).

Table 5

Results of Leslie depletion analyses on cod trawl and longline fishery data collected in the (**A**) high sampling-effort (HSE) survey area and (**B**) east of the survey area (Fig. 2). Dates when data were collected are listed, along with the regression parameters (q=slope and y-intercept=qB_0) and statistics (P=probability that slope is not significantly different from 0, r=Pearson correlation coefficient, and 95% confidence interval (CI) on B_0). For the trawl fishery in the HSE area, three different levels catch for the target fishery were used (20%, 40%, or 60% of the total catch per haul was cod). Cumulative catches in each area are defined as the catch from 1 January through the end of the period analyzed.

A High sampling-effort survey area

	Gear			
	Longline 13 Feb–24 Mar	Trawl 20% 6 Feb–24 Mar	Trawl 40% 6 Feb–24 Mar	Trawl 60% 6 Feb–24 Mar
Cumulative catch (t)	11,631	11,631	11,631	11,631
B_0 (t)	14,251	14,806	14,119	14,410
95% CI on B_0 (t)	9608–22,195	10,549–21,570	9526–21,942	8989–24,860
q	0.000115	0.000172	0.000207	0.000212
y-intercept	1.6395	2.5442	2.9246	3.0573
P	<0.000001	<0.000001	<0.000001	<0.000001
No. of days (n)	27	47	46	46
r^2	0.712	0.635	0.577	0.479

B East of survey area

	Gear	
	Longline 1 Jan–2 Mar	Trawl 20% 20 Jan–21 Mar
Cumulative catch (t)	6340	6837
B_0 (t)	14,671	
95% CI on B_0 (t)	10,934–20,936	
q	0.000053	
y-intercept	0.7707	
P	<0.000001	0.65
No. of days (n)	61	49
r^2	0.515	0.004

Although a portion of the AE of the sampling area is also critical habitat, the majority of it is not. Cod are thought to move from the areas east and south of the survey area to aggregate within critical habitat, particularly north of Unimak Island, for spawning (Shimada and Kimura, 1994; Thompson and Dorn, 2002). Leslie analyses were conducted on longline data collected from 1 January to 2 March in the AE of the survey area, and on trawl data collected from 20 January to 21 March. The longline data yielded a highly significant negative relationship between CPUE and cumulative catch (P<0.000001), whereas the trawl data did not (P=0.65; Table 5 and Fig. 6).

Trawl fishery CPUE in the HSE area was not correlated with daily average vessel length for the period 20 January–30 April 2001 (P=0.16; r^2=0.02; Fig. 8). The data from the analysis period 6 February–24 March are highlighted in Figure 8. Although there was a significant linear relationship between vessel length and CPUE for this shorter period (P=0.004), the correlation coefficient was low (r^2=0.16), indicating that daily average CPUE and vessel length were poorly correlated.

Discussion

The bottom trawl survey point estimate of cod biomass in the HSE area (31,312 t) is approximately twice the values derived from analyses of fishery data (approximately 14,500 t). This is in part because the fishery worked almost exclusively within the eastern two-thirds of the HSE area. Restratifying the HSE survey yields biomass estimates of 23,329 t for the eastern two-thirds used by the fishery and 7983 t for the western portion. The fishery-derived biomass estimates for the eastern portion of the HSE survey area are within the 95% confidence bounds on the survey estimate (12,846–33,812 t).

In addition, the survey biomass estimate for the eastern two-thirds of the HSE area is within or close to the upper 95% confidence bounds of the Leslie analyses of trawl and longline Pacific cod fishery data (Table 5).

One possible explanation for the lower fishery-derived estimates in the eastern portion of the HSE area is that emigration of fish after spawning contributed to the low CPUEs observed near the end of the fishery time series. If this emigration occurred, however, it went largely undetected in the neighboring areas. Emigration over the course of the fishery would decrease CPUEs faster than what would be attributable to fisheries alone, which would, in turn, decrease the estimate of initial biomass.

Plots of fishery CPUEs of Pacific cod were very similar for all gears used in each area. This finding indicates that these time series are useful as indices of relative cod abundance. Similarly, inferences can be made through analyses of fishery CPUE data regarding fish movement from area to area (or lack thereof) to a possible cause in the observed declines in CPUE (or local abundance). For instance, the lack of fishery CPUE increases in areas to the north, east, and south of the HSE survey area in March indicates that emigration was not a significant factor in the CPUE decline observed in both the longline and trawl fishery CPUE data from early February through 24 March. In fact, in the AE of the survey area through 2 March, longline CPUE declined, indicating that either fish left this area (to the north) or were reduced in abundance by fishing and were not replenished. Although the time series from the AS of the survey area is short, there is no indication that cod moved there in early March. There is also no evidence that cod moved north to the LSE survey area because the longline or pot fleets targeting cod did not move there, nor did the proportion of cod in trawl hauls increase (otherwise they would have been labeled as a cod-target fishery). It is possible that cod emigrating from the HSE area were so dispersed or their catchabilities were much lower than those for residents in other areas that their presence went undetected, but there is no evidence to suggest that either of these were any more likely than the more simple assumption that changes in CPUE within the fished area represented real changes in local abundance even after accounting for some level of emigration. If cod immigration exceeded emigration for the HSE area during early March as CPUEs were declining, then fishery-derived estimates of initial biomass calculated in our study are biased high.

Pot fishery CPUE data in the AS of the sampling area and in the HSE area indicated that there was an influx of Pacific cod from the south in mid-March. This was evident from the increase in pot fishery CPUE on 18 March in the AS of the survey area and beginning on 24 March in the HSE area. Cod may have moved into nearshore sections of the HSE area where they would be more vulnerable to pot gear than to trawlers. However, on 25–26 March, trawl CPUE on the border of the HSE area and the AE of the survey area increased substantially, indicating that these fish had moved offshore to areas worked by trawlers, or that they became highly aggregated (perhaps just prior to spawning). The late-March "pulse" of Pacific cod biomass was probably smaller than the

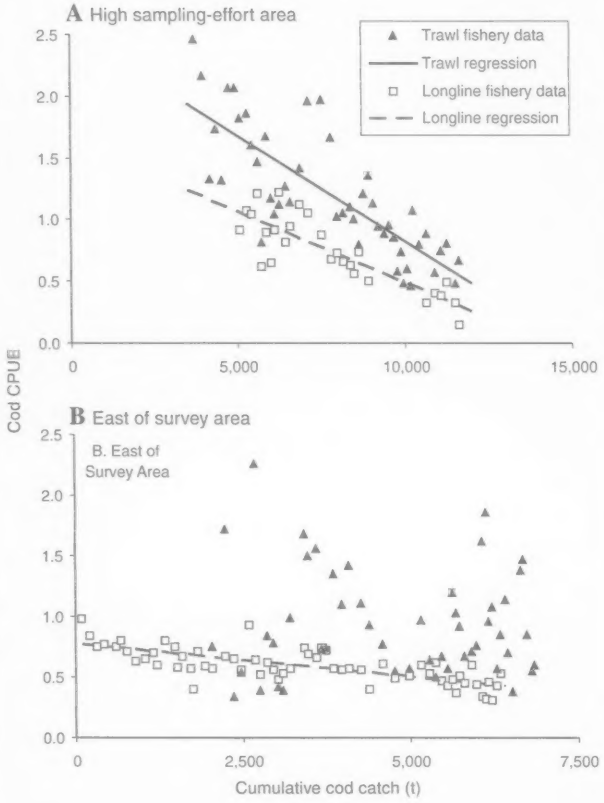

Figure 6

Daily average catch per unit of effort (CPUE) of Pacific cod (*Gadus macrocephalus*) by the observed Pacific cod fishery by gear type plotted against the estimated cumulative catch of cod by the groundfish fishery in the high sampling-effort area (**A**) and in the area east of the survey area (**B**; Fig. 1). For the trawl fishery (at least 20% of the haul catch was cod), CPUE = t/h; for the longline fishery, CPUE = t/1000 hooks. Lines are shown for those regressions whose slope was significantly different from 0 ($P<0.05$; Table 5).

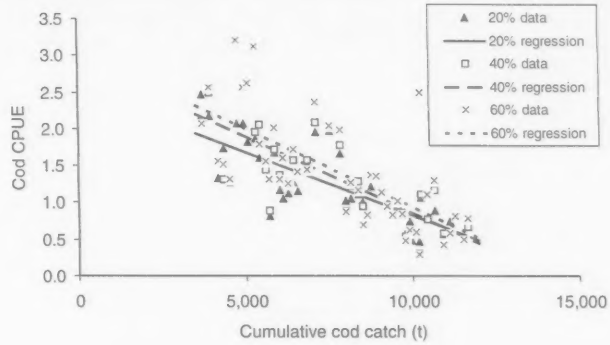

Figure 7

Catch per unit of effort (CPUE; t/h) of Pacific cod (*Gadus macrocephalus*) by the cod trawl fishery in the high sampling-effort area plotted against cumulative catch of cod in the same area by all groundfish fisheries. Three different levels of the cod fishery catch are used (20%, 40%, or 60% cod in each haul).

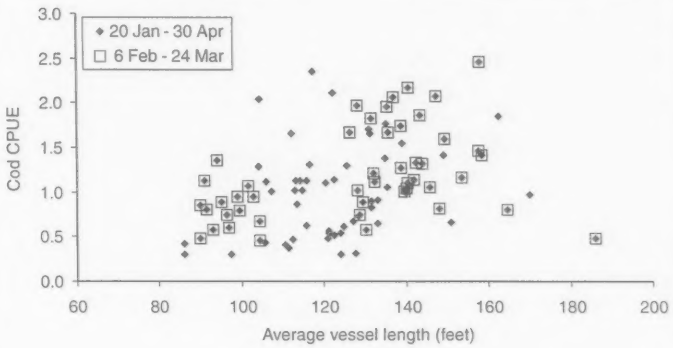

Figure 8

Daily average Pacific cod (*Gadus macrocephalus*) catch per unit of effort (CPUE; t/h) plotted against daily average vessel length for the trawl cod fishery in the high sampling-effort area in two time periods: 20 January–30 April 2001, and 6 February–24 March 2001.

initial influx that peaked in early February because it sustained the fishery for only 1–2 weeks, and resulted in cod catches of only approximately 7500 t from all four areas.

In the stock assessment for Pacific cod in the eastern Bering Sea and Aleutian Islands (BSAI; Thompson and Dorn, 2002), the estimate of age 3+ biomass in 2001 was approximately 1.284 million t, whereas the female spawning biomass was approximately 359,000 t. Doubling the latter to account for male spawner biomass, the survey and fishery data discussed in the present study indicate that only 4% of the adult spawning and 3% of the age 3+ biomass was in the HSE area, and only about 7% and 4%, respectively, in the entire area surveyed. The area north of Unimak Island is thought to be one of the principal spawning grounds for Pacific cod in the eastern Bering Sea (Shimada and Kimura, 1994; Thompson and Dorn, 2002). The results reported in the present study may indicate that either 1) this is not one of the principal spawning grounds for Pacific cod in the eastern Bering Sea and most spawning occurs elsewhere, 2) the stock assessment estimates are too high, or 3) Pacific cod aggregated in the area after the survey occurred.

Biomass estimates from the assessment are approximately twice those derived directly from bottom trawl

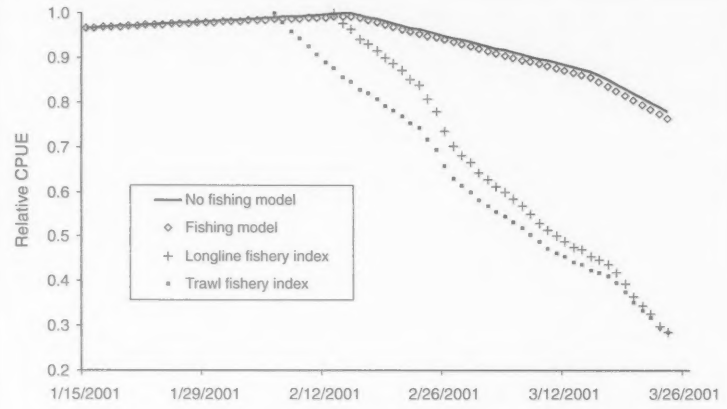

Figure 9

Comparison of relative abundance of Pacific cod (*Gadus macrocephalus*) in portions of Steller sea lion (*Eumetopias jubatus*) critical habitat from 15 January–24 March 2001 based on 1) no fishing model: the proportion of the maximum biomass (on 15 February) in critical habitat each day; 2) the fishing model: subtracting catch per day from 15 January–24 March 2001 in high and low sampling-effort areas from the no fishing model (total of 12,800 t); 3) longline fishery catch-per-unit-of-effort (CPUE) index of abundance from the high sampling-effort area, 13 February to 24 March (assigned a value of 1 on 13 February); and 4) trawl fishery (20% threshold) CPUE index of abundance from the high sampling-effort area, 6 February to 24 March (assigned a value of 1 on 6 February).

surveys of the entire Bering Sea shelf conducted in summer (Thompson and Dorn, 2002). This difference stems from highly domed-shaped selectivity-at-length schedules for the summer surveys and most fishery catches of cod (Thompson and Dorn, 2002). As a consequence, the model "assumes" that fewer cod are caught in proportion to their actual abundance at lengths greater than 45 cm for the survey catch and 80 cm for the fishery catch. However, it is unclear how large cod avoid capture during surveys or by longline, pot, and trawl fishery gear as implied by the dome-shaped selectivity-at-length schedules.

A seasonal model of Pacific cod movement patterns into and out of Steller sea lion critical habitat (Fig. 2) indicates that relative Pacific cod biomass inside critical habitat is highest in February, then drops 13% in March and 44% by April. If these values are assigned to the middle of each month and daily values are extrapolated linearly, the relative change from 15 February through 24 March is 23% (Fig. 9). Fishery indices of abundance in the HSE area in January and February are consistent with this seasonal pattern, with both trawl and longline CPUEs increasing from January to February. According to Figure 2 and the 2001 age 3+ biomass estimate (Thompson and Dorn, 2002), catches through 24 March within the entire survey area (12,806 t) represented only 1% of the BSAI stock and should have reduced the relative biomass of cod within critical habitat by only an additional 2%. Thus, the total reduction in relative cod biomass within critical habitat from mid-February through late March after accounting for fishing and emigration should have been 25% (Fig. 9). Longline and trawl fishery CPUE data in the HSE area provide an independent estimate of relative cod biomass. Both indices indicate that the reduction in relative cod biomass within the HSE survey area through 24 March was 71–46% greater than that predicted by the model.

Catches and biomass estimates of Pacific cod for different time periods and areas can be used to compute harvest indices (catch divided by observed biomass). For instance, the harvest index within the entire survey area (based on the catch from 1 January through 24 March and the survey biomass estimate) was 26% (12,806 or÷49,032). If the focus is narrowed to only the HSE survey area through 24 March, the harvest index was 37% (11,631 or÷31,312). However, both the fish and the fishery were concentrated within the HSE area. The eastern two-thirds of the HSE survey area had survey and fishery-derived biomass estimates of 23,418 t and ~14,500 t, respectively. With the area of fishery effort more precisely defined, local harvest indices increase even further, ranging from 50% (11,631 or÷23,329) to 80% (11,631 or÷14,500).

The annual harvest rate of BSAI cod in 2001 was estimated to be approximately 11% (Thompson and Dorn, 2002). The total catch of cod in the BSAI through 24 March represented only 44% of the total catch of Pacific cod in 2001. Therefore, the harvest rate through 24 March should only have been 44% of 11%, or about 5%. The local harvest indices estimated in the present study, which ranged from 26% to 80%, were five to 16 times greater than that on the BSAI Pacific cod stock as a whole in 2001. Much of the area used by the fishery is designated as critical habitat for the endangered Steller sea lion, primarily because of the prey resources available within it. In addition, the fisheries occurred in the winter and early spring, when sea lions are most likely to consume Pacific cod (Sinclair and Zeppelin, 2002). It is not known how or if cod fishery catches in this area affect Steller sea lion foraging success. One objective of the Pacific cod fishery management regulations is to minimize the competitive interactions between locally intense fisheries and Steller sea lions. The suite of groundfish fishery regulations enacted in 2001 and 2002 work together to avoid adverse modification of critical habitat under the ESA. However, based on the observations during 2001 discussed in the present study, regulations for the eastern Bering Sea Pacific cod fishery should be reviewed to ensure that they meet these management objectives.

Acknowledgments

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Mitochondrial gene sequences useful for species identification of western North Atlantic Ocean sharks

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Abstract—Molecular-based approaches for shark species identification have been driven largely by issues specific to the fishery. In an effort to establish a more comprehensive identification data set, we investigated DNA sequence variation of a 1.4-kb region from the mitochondrial genome covering partial sequences from the 12S rDNA, 16S rDNA, and the complete valine tRNA from 35 shark species from the Atlantic fishery. Generally, within-species variability was low in relation to interspecific divergence because species haloptypes formed monophyletic groups. Phylogenetic analyses resolved ordinal relationships among Carcharhiniformes and Lamniformes, and revealed support for the families Sphyrnidae and Triakidae (within Carcharhiniformes) and Lamnidae and Alopidae (within Lamniformes). The combination of limited intraspecific variability and sufficient between-species divergence indicates that this locus is suitable for species identification.

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Seventy-three species of sharks inhabit the United States territorial waters of the Atlantic Ocean, Gulf of Mexico, and Caribbean Sea (Compagno, 1984a, 1984b). All but one (spiny dogfish, *Squalus acanthias*, managed separately) are managed under the current Fisheries Management Plan (FMP) for highly migratory species (NMFS[1]). Thirty-three species are of lesser commercial importance and are relegated to the "deepwater and other" species management group, and 19 species cannot be landed commercially or recreationally ("prohibited species" group). The remaining 20 species are of interest to the commercial shark fishery and are categorized as large coastal species (LCS), small coastal species (SCS), and pelagic species management units in the current FMP. Although these management units are practical, it is clear that species respond uniquely to exploitation and therefore should be managed on a species-by-species basis (Castro et al., 1999; NMFS[2]). Species-level management is widely recommended (e.g., FAO Marine Resource Service, 2000) but is complicated by the paucity of species-specific fisheries data, stemming, in part, from an inability to accurately identify species.

Many commercially important species (e.g., within Carcharhiniformes) are difficult to identify whole, and this task is more daunting if individuals are processed (head, entrails, and fins are removed); unfortunately, at-sea processing is widespread in the industry (Castro[3]). Although current U.S. legislation prohibits the practice of "finning" (where fins are retained and carcasses are discarded at sea),

[1] NMFS (National Marine Fisheries Service). 2003. Final amendment 1 to the fishery management plan for Atlantic tunas, swordfish and sharks, 599 p. Office of Sustainable Fisheries, Highly Migratory Species Management Division, NMFS, NOAA, 1315 East West Highway, SSMC3, Silver Spring, MD 20910.

[2] NMFS (National Marine Fisheries Service). 2001. Final United States national plan of action for the conservation and management for sharks, 90 p. Office of Sustainable Fisheries, Highly Migratory Species Management Division, NMFS, NOAA, 1315 East West Highway, SSMC3, Silver Spring, MD 20910.

[3] Castro, J. I 1993. A field guide to the sharks commonly caught in commercial fisheries of the southeastern United States. NOAA Tech. Memo. NMFS-SEFSC-338, 47 p. Southeast Fisheries Science Center, NMFS, NOAA, 75 Virginia Beach Dr., Miami, FL 33149.

the landing of fins is allowed where carcasses and fins are off-loaded at the same time in a no more than 1:20 (fin-to-carcass) weight ratio. However, serious problems can arise in matching off-loaded fins to processed carcasses. In and of itself, the landing of shark fins can be lucrative; fins accounted for more than 50% of the total Atlantic shark fishery value in 2002 (NMFS[4]). Because preferences exist for fins from certain species, exvessel prices for specific types of fin vary considerably (e.g., Weber and Fordham, 1997). It is perhaps not surprising that augmenting the fin-to-carcass ratio with spoiled meat or "finning" target species out of season (and subsequently attributing the fins to fish that are allowed to be caught during the season) might not be uncommon (Vannuccini, 1999). Clearly, these possibilities lead to the challenge of matching collected fins to processed carcasses. Therefore, accurate and reliable species identification methods are paramount for law enforcement and sound species management.

Molecular species identification research on sharks has been driven largely by resolution of specific problems associated with the fishery. For example, Heist and Gold (1999) used mtDNA sequence data to develop restriction fragment assays that differentiate 11 species of carcharhiniform sharks commonly encountered in the LCS fishery. Similarly, Pank et al. (2001) used multiplex PCR to differentiate two morphologically similar shark species (*Carcharhinus obscurus* and *C. plumbeus*)—an approach that was expanded by Shivji et al. (2001) to include five additional species (with some overlap of species included by Heist and Gold 1999). Both approaches are relatively rapid, inexpensive, and easily implemented; however, they appear most applicable when the number of species investigated is limited. In sum, of the thirty-nine species of sharks that are not in the "deepwater and other" management group, molecular species identification assays have been developed for fifteen species (9 LCS, 3 pelagic, and 3 in the prohibited species management groups) (Heist and Gold, 1999; Pank et al., 2001; Shivji et al., 2001), leaving 24 species without molecular methods for identification.

Some investigators have instead turned to DNA sequence analysis to resolve issues of species identification (Takeyama et al., 2001; Akimoto et al., 2002; Jerome et al., 2003). This approach is exemplified best by the recent development of computer interfaces that allow access to and analysis of large DNA databases (DNA Surveillance, Ross et al., 2003; ARB, Ludwig et al., 2004). Simply put, these databases circumvent the tedious process of scanning large taxonomically diverse DNA repositories (e.g., GenBank) by allowing the user to access (DNA Surveillance) or maintain (ARB) taxonomically restricted sets of reference sequences. Users can submit "unknown" sequences to compare against specified sequence subsets; subsequent analyses are returned as genetic distances (between unknown and reference sequences) and include a phylogenetic hypothesis.

The power of this approach lies in the ease with which reference sequences can be added to the database, in the "quality-control" that can be exerted over subsequent additions to the reference sequences, and in the ease with which geographic variation within species can be included. The success of this approach, however, hinges on the information contained in the gene in the reference database. The inception of this approach, as applied to commercially important sharks, requires a sufficiently informative set of reference sequences against which searches can be made. The aforementioned molecular approaches (RFLP, multiplex PCR) include a diversity of gene regions (mitochondrial DNA, nuclear ITS); thus no comprehensive data set exists for commercially landed Atlantic shark species. Fortunately, recent work with a 2.4-kb fragment of the mitochondrial genome (spanning 12S rDNA to 16s rDNA) to examine the phylogenetic relationships among shark orders has shown that this region may be useful in resolving relationships at this taxonomic level (Douady et al., 2003). Unfortunately, sampling within orders was limited, and it is thus unknown whether this region contains sufficient phylogenetic signal at lower taxonomic levels.

We present here mtDNA sequence data of a smaller fragment of the same region containing partial sequence information for the mitochondrial 12S rDNA, 16S rDNA, and the complete valine tRNA from 35 shark species (including all 20 commercially exploited species, 12 of 19 prohibited species, the spiny dogfish, and two species of *Mustelus*). We suggest that a suitable locus for species-identification purposes will permit identification of unequivocally distinct species (i.e., large genetic differentiation between species compared to within species) and offer the potential for meaningful phylogenetic comparisons (important when "query" animals are absent or not adequately represented in a molecular database). Keeping in mind issues of species identification and fisheries management, we examine this mtDNA region for patterns of genetic variability and assess its utility in phylogenetic reconstruction. We then discuss the use of this region for the underpinnings of a validated reference DNA database suitable for forensic and fisheries management applications.

Methods

Sample collection

Voucher Atlantic Ocean shark samples (muscle, fin, or blood) were obtained from the CCEHBR Marine Forensics archive in Charleston, SC (Table 1). Samples were accompanied by species certification and chain-of-custody forms. Muscle and fin samples were either frozen at

[4] NMFS (National Marine Fisheries Service). 2003. Stock assessment and fishery evaluation report for Atlantic highly migratory species (SAFE), 274 p. Office of Sustainable Fisheries, Highly Migratory Species Management Division, NMFS, NOAA, 1315 East West Highway, SSMC3, Silver Spring, MD 20910.

Table 1

Scientific and common names of samples, number of individuals sampled (n), species codes, and Genbank accession numbers. Taxonomy follows Campagno (1984, 2001). Species codes correspond to a representative individual in the National Ocean Service Marine Forensics Program (CCEHBR, Charleston, SC) tissue archive with that particular haplotype (except for *Heterodontus francisci* Hfra1).

Order	Family and species	Common name	Code (n)	Accession
Carcharhiniformes	Carcharhinidae			
	Carcharhinus acronotus	Blacknose	Cacr003(3)	AY830721
	C. altimus	Bignose	Calt001(2)	AY830722
	C. brevipinna	Spinner	Cbre001(3)	AY830723
	C. falciformis	Silky	Cfal003(1)	AY830725
			Cfal006(1)	AY830726
	C. isodon	Finetooth	Ciso004(1)	AY830727
			Ciso010(1)	AY830728
			Ciso015(1)	AY830729
	C. leucas	Bull	Cleu003(3)	AY830730
	C. limbatus	Blacktip	Clim004(1)	AY830731
			Clim006(2)	AY830732
	C. longimanus	Oceanic whitetip	Clon000(1)	AY830736
			Clon002(1)	AY830733
			Clon005(1)	AY830734
			Clon006(1)	AY830735
	C. obscurus	Dusky	Cobs000(1)	AY830737
			Cobs001(3)	AY830738
	C. perezi	Caribbean reef	Cper001(2)	AY830739
			Cper002(2)	AY830740
	C. porosus	Smalltail	Cpor001(1)	AY830743
	C. plumbeus	Sandbar	Cplu004(2)	AY830741
			Cplu023(1)	AY830742
	C. signatus	Night	Csig002(1)	AY830744
	Galeocerdo cuvier	Tiger	Gcuv003(3)	AY830746
	Negaprion brevirostris	Lemon	Nbre005(1)	AY830756
	Prionace glauca	Blue	Pgla004(1)	AY830760
			Pgla0020(1)	AY830761
			Pgla0022(1)	AY830762
	Rhizoprionodon terraenovae	Sharpnose	Rter001(2)	AY830763
			Rter026(1)	AY830764
	Sphyrnidae			
	Sphyrna lewini	Scalloped hammerhead	Slew003(2)	AY830768
	S. mokarran	Great hammerhead	Smok003(3)	AY830769
	S. tiburo	Bonnethead	Stib016(2)	AY830770
			Stib018(1)	AY830771
	S. zygaena	Smooth hammerhead	Szyg681(6)	AY830772
	Triakidae			
	Mustelus canis	Smooth dogfish	Mcan003(3)	AY830754
	M. norrisi	Florida smoothhound	Mnor001(2)	AY830755
Lamniformes	Alopiidae			
	Alopias superciliosus	Bigeye thresher	Asup001(1)	AY830718
			Asup006(1)	AY830719
	A. vulpinus	Thresher	Avul002(1)	AY830720
	Lamnidae			
	Carcharodon carcharias	White	Ccar002(3)	AY830724
	Isurus oxyrinchus	Shortfin mako	Ioxy005(1)	AY830747
			Ioxy032(1)	AY830748
			Ioxy051(1)	AY830749
	I. paucus	Longfin mako	Ipau002(2)	AY830750
			Ipau005(1)	AY830751
	Lamna nasus	Porbeagle	Lnas001(2)	AY830752
			Lnas003(1)	AY830753

continued

Table 1 (continued)

Order	Family and species	Common name	Code (*n*)	Accession
	Odontaspididae			
	Carcharius taurus	Sand tiger	Otau004(1)	AY830757
			Otau005(1)	AY830758
			Otau007(1)	AY830759
Orectolobiformes	Ginglymostomatidae			
	Ginglymostoma cirratum	Nurse	Gcir001(2)	AY830745
Hexanchiformes	Hexanchidae			
	Hexanchus vitulus	Bigeye sixgill	Hvit1(1)	AY830716
	Heptranchias perlo	Sevengill	Hper1(1)	AY830715
Squaliformes	Squalidae			
	Squalus acanthias	Spiny dogfish	Saca002(1)	AY830765
			Saca003(2)	AY830766
Squatiniformes	Squatinidae			
	Squatina dumeril	Atlantic angel	Sdum001(3)	AY830767
Heterodontiformes	Heterodontidae			
	Heterodontus francisci	Horn shark	Hfra(1)	NC003137

–80°C, dried, or stored in 70% EtOH. Blood was stored at room temperature in sodium dodecyl sulfate-urea (SDS-urea: 1% SDS, 8M urea, 240 mM Na_2HPO_4, 1mM EDTA pH 6.8). Total nucleic acids were extracted from frozen, dried, and EtOH-preserved samples by using DNeasy Tissue Kits and following manufacturer's recommendations (Qiagen, Valencia, CA). DNA was isolated from blood in SDS-urea according to White and Densmore (1992; protocol 11). Extracted DNA was visualized by electrophoresis in a 1% agarose gel stained with 0.4 ng/mL of ethidium bromide in 1× Tris-borate-EDTA (TBE: 89 mM Tris-borate, 2 mM Na_2EDTA, pH 8). A 1-kb DNA ladder (Promega, Madison, WI) was used as a size standard.

Amplification and sequencing

Primers 12SA-5′ and 16SA-3′ (Palumbi, 1996) were used to amplify an approximately 1400-bp region spanning the 3′ end of the 12s rDNA, the valine tRNA, and the 5′ end of the 16s rDNA region of mitochondrial DNA (mtDNA). Samples were amplified in 50 µL reactions containing ~50 ng of template DNA, 20 mM Tris-HCl pH 8.4, 50 mM KCl, 0.2 mM each dNTP, 2 mM $MgCl_2$, 20 mM each primer, and 2.5 units *Taq* DNA polymerase (Gibco BRL, Rockville, MD). Thermal cycling consisted of an initial denaturation at 94°C for 1.5 minutes, followed by 30 cycles of 40 seconds at 94°C, 40 seconds at 52°C, and 50 seconds at 72°C, and a final extension step of 15 minutes at 72°C. Negative controls (no template) were included in each set of reactions. PCR products were gel-purified as described in Rosel and Block (1996) and 20–50 ng were used as template for ABI Big Dye Terminator (v. 1.0, Applied Biosystems, Foster City, CA) cycle sequencing reactions. Sequence was obtained with amplification primers 12SA-5′, 16SA-3′ and two additional internal sequencing primers. Sequencing reaction products were precipitated with ethanol, washed according to sequencing kit instructions, dried in a Savant Speedvac Plus, and resuspended in 4 µL of loading dye (5:1 Hi-Di formamide:dextran blue). Fragments were analyzed on an Applied Biosystems 377 automated DNA sequencer.

Sequence analysis and alignment

Sequences were edited with SEQUENCHER (vers. 3.0; Gene Codes Corp., Detroit, MI). We included three additional sequences from GenBank: horn shark (*Heterodontus francisci*, NC003137) to represent the family Heterodontidae, thorny skate (*Raja radiata*, AF106038), rabbit fish (*Chimaera monstrosa*, AJ310140), and the Atlantic guitarfish (*Rhinobatis lentiginosus*, AY830717—this study) to serve as outgroups for phylogenetic analyses. Sequences were aligned by using a linear hidden Markov model (HMM) as implemented in SAM (Sequence Alignment and Modeling System; Hughey and Krogh, 1996; Karplus et al., 1998) with default settings. The alignment file is available from the senior author.

Phylogenetic hypotheses were constructed by using the maximum parsimony (MP) and neighbor-joining (NJ) algorithms implemented in PAUP 4.0b10 (Sinauer Associates, Sunderland, MA). NJ analyses employed a variety of pairwise distance measures, but the distance measure used had little or no effect on the recovered topologies. Phylogenies recovered with MP with equally weighted characters were generally concordant with those recovered by NJ, particularly when bootstrap consensus trees were compared. For ease of interpretation, we report NJ analyses using p-distances as a metric. Bootstrapping (Felsenstein, 1985) was used to estimate the reliability of NJ reconstructions (1000 pseudoreplicates).

Results

Sequence variation and divergence

An approximately 1.4-kb gene region was amplified and sequenced from 93 samples representing 35 shark species. Fifty-seven of the 93 individuals had unique haplotypes (Table 1, Fig. 1). An alignment of these haplotypes with several outgroups with the SAM algorithm resulted in a 1510 position consensus alignment after the introduction of gaps. Of these 1510 aligned positions, 717 positions were variable and 543 were parsimony informative. Transition outweighed transversion substitutions by a factor of 4.27. Considering only phylogenetically informative sites within the ingroup, we found that nucleotide composition did not differ significantly among haplotypes (A: 35.9%, C: 21.9%, G: 16.9%, T: 25.3%; χ^2=175.6, P=0.39).

Phylogenetic analysis

Unweighted parsimony analysis produced 24 equally parsimonious trees of length 2733 (CI=0.39, RI=0.74) that differed primarily in the relationships among haplotypes within species (not shown). Neighbor-joining analyses produced nearly identical topologies regardless of the distance metric used. When differences were noted, they often involved trivial placements of individual variants within species or the placement of branches that were poorly supported by bootstrap analyses regardless of the reconstruction method employed. For this reason, we present phylogenetic hypotheses generated by neighbor-joining, using p-distances as a surrogate for all analyses.

Most clades containing multiple haplotypes within species were highly supported by bootstrap analyses. Of 16 species represented by more than a single sequence, 15 were recovered as monophyletic groups in 100% of 1000 bootstrap replicates (Fig. 1). Sequence divergence within species was generally trivial compared to among-species divergences. For example, sequence divergence among haplotypes within species of *Carcharhinus* differed by approximately two orders of magnitude from that among species within the genus (average p-distance of 0.05% and 4.16%, respectively). The exception involved haplotypes observed within *C. plumbeus* that were supported as monophyletic by fewer than 70% of 1000 bootstrap replicates in MP and NJ analyses. Interestingly, a sister group relationship between *C. plumbeus* and *C. altimus* was highly supported by bootstrapping, and average sequence divergence within species (0.14%) was only about one-third of that observed between these two (0.43%).

Some higher order relationships were recovered with high bootstrap support. Notably the Carcharhiniformes were strongly supported as monophyletic, as were the families Sphyrnidae and Triakidae. The family Carcharhinidae was poorly supported as monophyletic, although a group that included *Negaprion*, *Prionace*, and all *Carcharhinus* was observed in a large number of bootstrap replicates. *Carcharhinus* was paraphyletic in the NJ topology, and *Negaprion* was nested within the genus, but this relationship received little support from bootstrapping. The Lamniformes were monophyletic and strongly supported by bootstrapping. Within this order, only the family Lamnidae received strong support, whereas support for a monophyletic Alopidae was moderate. The order Hexanchiformes was recovered as a monophyletic group; however bootstrap support for this grouping was low.

Discussion

Our goal was to assess whether the 12s–16s region of the shark mitochondrial genome contained sufficient genetic variation and phylogenetic signal to be useful in species identification. Of the 35 species examined, 6 species were each represented by a single individual, and 16 of the remaining 29 species contained variants at the mtDNA locus examined. Importantly, all within-species variants formed strongly supported monophyletic groups concordant with morphologically based species descriptions. Intraspecific variability was low in relation to interspecific divergence at this locus and in no instance was a paraphyletic relationship between species observed. The combination of limited intraspecific variability combined with sufficient between-species divergence indicates that this locus is suitable for species identification.

Two exceptions to this generalization of low within versus large between-species differentiation exist in our phylogenetic hypothesis—one involving the sister species pair *C. plumbeus* and *C. altimus*. In an alignment of mitochondrial sequences from these species, only 5 or 7 transition substitutions were observed across approximately 1.4 kb of sequence data. Interestingly, Heist and Gold (1999) included these two taxa in their cytochrome-*b* RFLP analysis, and again, Atlantic samples of *C. plumbeus* and *C. altimus* differed by only a single transition in 395 basepairs (0.25%), and there were more substitutions observed between Atlantic and Pacific *C. plumbeus* than between Atlantic samples of *C. plumbeus* and *C. altimus* (Table 2 in Heist and Gold 1999). The next most closely related pair of taxa in our phylogenetic hypothesis comprised two other Carcharhiniforms, *C. longimanus* and *C. obscurus*, a taxon pair differing by approximately 1.44% sequence divergence, compared with an average of 0.06% within taxon diversity. These two taxa were considered by Shivji et al. (2001) while developing a multiplex PCR assay for six commercially important pelagic species. Specifically, assays developed to diagnose *C. obscurus* could not discriminate between *C. obscurus* and *C. longimanus*, two closely related species in our phylogenies. The *C. plumbeus* and *C. altimus* species pair was not considered by Shivji et al. (2001); thus no comparison to the Heist and Gold (1999) cytochrome-*b* sequence/RFLP or the 12s–16s data set presented in our study was possible. We are currently analyzing additional samples,

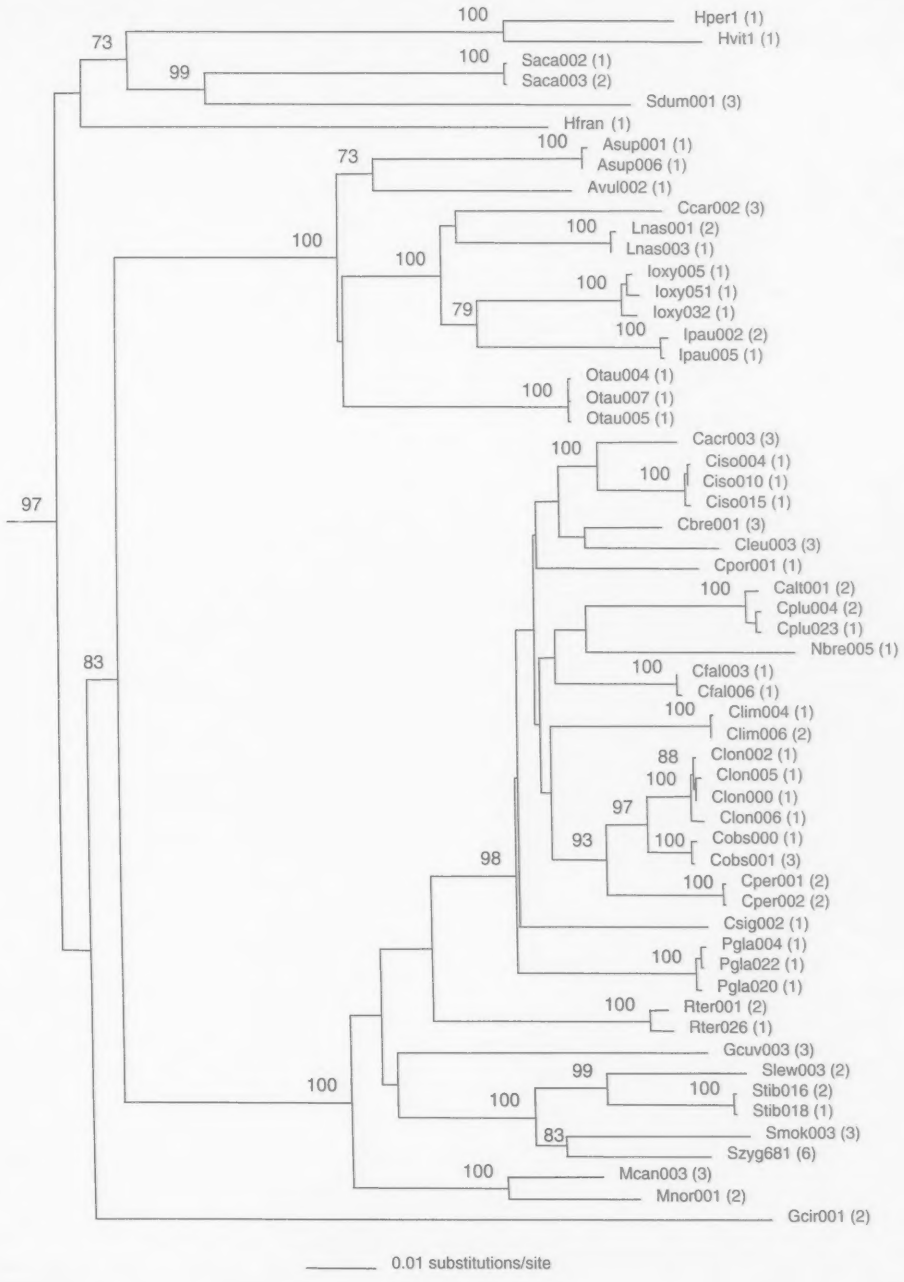

Figure 1

Neighbor-joining tree showing relationship of observed 12s–16s haplotypes among 36 species of shark. Codes are defined in Table 1 and numbers in parentheses indicate the number of individuals found with the indicated haplotype. Bootstrap support is indicated as numbers immediately above the relevant node (only values greater than 70% are shown). The phylogeny was rooted with several outgroup taxa (*Heterodontus francisci* (NC003137), *Raja radiata* (AF106038), *Chimaera monstrosa* (AJ310140), and *Rhinobatis lentiginosus* (AY830717)).

including a more comprehensive geographical survey of these four species to confirm that the genetic differences observed are diagnostic. However, it is clear that DNA sequence-based approaches appear more powerful in discriminating closely related species pairs and less likely to produce false positives than other DNA-based assays.

Although it was not our intent to conduct an exhaustive analysis of higher-order relationships among western North Atlantic shark species, some interesting results nonetheless deserve mention. First, the orders Carcharhiniformes and Lamniformes were strongly supported as monophyletic, as were the families Sphyrnidae, Triakidae, and Lamnidae that were included in the study. The order Hexanchiformes was likewise monophyletic, but bootstrap support for this grouping was low. The family Carcharhinidae was poorly supported as monophyletic, which is consistent with previous studies (Nalyor, 1992; Nelson, 1994; Musick et al., 2004). Interestingly, our phylogenetic hypotheses place the family Triakidae basal to all other families within the Carcharhiniformes, following Compagno (1988), but this position was not strongly supported and is predicated on limited sampling of Carcharhiniform familes (only four of eight were included in our analysis). Clearly this gene region contains some phylogenetically useful information regarding shark relationships, confined principally to higher-level groupings.

We are careful in judging the utility of a locus for species identification on the basis of phylogenetic signal alone. Clearly, rapidly evolving molecular markers are valuable tools for species identification but might not be appropriate for reconstructing phylogenetic relationships at certain scales. Conversely, those regions containing sufficient signal to generate reasonable phylogenetic reconstructions (i.e., general concordance with accepted phylogenetic relationships based on other independent characters) must be useful (and appropriate) markers for species identification. Further, these regions are amenable to the addition of uncharacterized species and the inclusion of intraspecific diversity (e.g., diverged mtDNA lineages within species). Importantly, however, DNA sequence-based approaches offer the potential to assign at least some level of taxonomic characterization to unknown or unrepresented samples. Although the use of DNA sequencing has historically been viewed as cost prohibitive, the genomic revolution over recent years has spawned cost-effective sequencing services, making routine sequencing of samples for species identification not only practical but optimal.

The size of the amplification product in the present study might place limitations on the application of this method to the poor-quality tissue and DNA often encountered in forensic studies. It has been our experience that the primers used in our study consistently have generated strong amplification products with DNA isolated from a variety of tissue types, including dried tissue and fins; however, we have yet to explore the range of amplifications possible using tissues more commonly encountered in forensic cases. To circumvent potential problems with large amplifications on degraded DNA samples, we have constructed a preliminary, searchable DNA-sequence database using the FASTA program (Univ. Virginia, Charlottesville, VA; Pearson, 1999) and the 12s–16s sequences presented in the present study. Our preliminary analyses indicate that all species examined in the study can be uniquely identified from approximately 400 bp of sequence generated by the 12SA-5′ primer. We are examining the limitations of sequence length in combination with the search accuracy of this informative fragment.

We are mindful of the restriction placed on these analyses due to limited within-taxon sampling (particularly within-family) and of the incomplete representation (notably the Pristophoriformes) of all orders of sharks and are aware that the phylogenetic affinities presented in this study could change with the addition of characters and taxa. These caveats notwithstanding, we believe that a taxonomically restricted DNA sequence database offers certain advantages over perhaps more rapid RFLP or multiplex PCR assays. DNA databases 1) can be "curated" (additions and access to the database can be selective) and distributed as an alignment suitable for further subsequent statistical or phylogenetic manipulation; 2) can be easily amended to include additional taxa, genetic variation within species, and additional gene loci more appropriate at various taxonomic scales; 3) allow for unequivocal assignment (subject to limits of discrimination of those loci included) of species identification while making available the raw data necessary for the development of more rapid assays (RFLP/Multiplex PCR) for select taxa (note that the opposite is not necessarily true); and, 4) facilitate the identification of those taxa not currently represented in the database through phylogenetic analysis.

In summary, we have found that the sequence of the 12S–16S region of the mtDNA that we examined contains ample information for discriminating between the shark species studied and shows promise for the placement of species not yet examined within the correct phylogenetic group (family). We are continuing to examine geographic variation within and among species and to assay genetic variability at nuclear loci in an effort to resolve potential introgression and (or) hybridization events. As information is added to our database, either in the form of additional species or loci, our species identification method will become more robust.

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Genetic variation of rougheye rockfish (*Sebastes aleutianus*) and shortraker rockfish (*S. borealis*) inferred from allozymes

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Abstract—Rougheye rockfish (*Sebastes aleutianus*) and shortraker rockfish (*Sebastes borealis*) were collected from the Washington coast, the Gulf of Alaska, the southern Bering Sea, and the eastern Kamchatka coast of Russia (areas encompassing most of their geographic distribution) for population genetic analyses. Using starch gel electrophoresis, we analyzed 1027 rougheye rockfish and 615 shortraker rockfish for variation at 29 protein-coding loci. No genetic heterogeneity was found among shortraker rockfish throughout the sampled regions, although shortraker in the Aleutian Islands region, captured at deeper depths, were found to be significantly smaller in size than the shortraker caught in shallower waters from Southeast Alaska. Genetic analysis of the rougheye rockfish revealed two evolutionary lineages that exist in sympatry with little or no gene flow between them. When analyzed as two distinct species, neither lineage exhibited heterogeneity among regions. *Sebastes aleutianus* seems to inhabit waters throughout the Gulf of Alaska and more southern waters, whereas *S.* sp. cf. *aleutianus* inhabits waters throughout the Gulf of Alaska, Aleutian Islands, and Asia. The distribution of the two rougheye rockfish lineages may be related to depth where they are sympatric. The paler color morph, *S. aleutianus*, is found more abundantly in shallower waters and the darker color morph, *Sebastes* sp. cf. *aleutianus*, inhabits deeper waters. *Sebastes* sp. cf. *aleutianus*, also exhibited a significantly higher prevalence of two parasites, *N. robusta* and *T. trituba*, than did *Sebastes aleutianus*, in the 2001 samples, indicating a possible difference in habitat and (or) resource use between the two lineages.

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Information about the biology and population dynamics of rougheye rockfish (*S. aleutianus*) and shortraker rockfish (*S. borealis*) is limited, and uncertainty exists about current stock abundance and long-term productivity. As adults, these two species are similar in appearance, have the same zoogeography, and share the same habitat. They were classified as a single species, *S. aleutianus* (Jordan and Evermann, 1898), until Barsukov (1970) described *S. borealis*. Tsuyuki and Westrheim (1970) also described *S. borealis* that same year (initially as *S. caenaematicus*), using biochemical methods. The distribution of rougheye rockfish is reported from Japan to southeastern Kamchatka (excluding the Sea of Okhotsk), to Navarin Canyon in the Bering Sea, throughout the Aleutian Islands, and south to San Diego, California (Tokranov and Davydov, 1997). Shortraker rockfish has a similar distribution; however, this species is much more abundant than rougheye rockfish in Russia—eastern Russian *Sebastes* biomass was composed of more than 90% shortraker and less than 1% rougheye rockfish for most regions, excepting the Commander Islands (Tokranov and Davydov, 1997). Both species have been reported at depths to 875 m (Allen and Smith, 1988), although longline (Sigler and Zenger[1]) and trawl surveys (NMFS triennial groundfish survey) indicate they are most abundant on the upper continental slope at 300–400 m depths. Krieger and Ito (1999) found the two species difficult to distinguish visually when viewed from a submersible but believed that the highly sedentary adults of both species share the same habitat, preferring substrates of sand or mud and frequent boulders and steep slopes.

Rougheye and shortraker rockfish are highly prized commercially but are particularly sensitive to overexploitation because of slow growth, late maturation, and long life spans. Half of rougheye rockfish are mature at 20

[1] Sigler, M. F., and H. H. Zenger Jr. 1994. Relative abundance of Gulf of Alaska sablefish and other groundfish based on the domestic longline survey, 1989. U. S. Dep. Commer., NOAA Tech. Memo. NMFS-AFSC-40, 79 p. Auke Bay Laboratory, 11305 Glacier Hwy., Juneau, AK 99801.

years of age (McDermott, 1994). Rougheye rockfish have been estimated to attain ages in excess of 200 years and shortraker rockfish in excess of 150 years (Munk, 2001). These two species are currently managed together as the "shortraker-rougheye" assemblage within waters managed under a North Pacific Fishery Management Council (NPFMC) fishery management plan. Commercial catch levels in NPFMC areas of the Bering Sea, the Aleutian Islands, and the Gulf of Alaska averaged 2400 t each year from 1999 to 2001 (Heifetz et al., 2002; Spencer and Reuter, 2002).

The annual catch quota for rockfish and most groundfish managed by the NPFMC is apportioned among five relatively large geographic areas: the eastern, central, and western Gulf of Alaska, the Aleutian Islands, and the eastern Bering Sea. Previous work in the Gulf of Alaska has indicated geographical segregation of the two rougheye species (Moles et al., 1998; Hawkins et al.[2]). Based on earlier designations of the International North Pacific Fisheries Commission, area boundaries have little biological basis. If the population structure of a particular species has different geographic boundaries than the boundaries of the designated management areas for the species, there is risk of over-harvest. The objective of this study is to examine the population structure of rougheye and shortraker rockfish by using allozyme variation. This is the first population structure study of these two species that encompasses all the North Pacific management areas and most of their biological ranges.

Methods

Collection

Adult rougheye rockfish were collected with bottom trawls from the Gulf of Alaska in 1993, the eastern Bering Sea in 1994, and from the Washington coast in 1998. They were also collected by longline from waters north of Unalaska Island in the Aleutian Islands in 1996, the central Gulf of Alaska and the northwestern Bering Sea near Russia in 1997, and north of Unalaska Island (Aleutian Islands) and in the eastern and central Gulf of Alaska in 2001. Shortraker rockfish were collected with bottom trawls from the Gulf of Alaska in 1993, the eastern Bering Sea in 1994, and by longline in the northwestern Bering Sea near Russia in 1997. Dates, locations, and sample sizes are reported in Table 1 and Figure 1.

Approximately 2–3 mL of liver, heart, and muscle were taken from each fish, temporarily stored in either a freezer (–20°C) or in liquid nitrogen, shipped to the Auke Bay Laboratory, Alaska, and stored at –80°C. Eye tissue was taken from the 1993 Southeast Alaska samples but was not collected during subsequent sampling efforts because initial experimentation yielded limited results from this tissue. Samples of heart tissue were sent to the University of Alaska for DNA analysis. Only liver tissue was taken from the Shumagin and Aleutian Islands rougheye rockfish samples in 2001 (regions 9b, 14a, and 16a). The right gill arch and a 4-inch section of the gut were sampled for parasite analysis from the rougheye rockfish 2001 Gulf of Alaska samples. These fish were also photographed, preserved in 10% formalin, and shipped to the Alaska Fisheries Science Center for future morphological studies.

Laboratory analysis

Protein enzymes from each sample were separated by horizontal starch-gel electrophoresis as described by Aebersold et al. (1987). Enzymes were screened by staining eye, heart, liver, and muscle tissue on each of six buffer systems (Table 2) by using general staining procedures (Harris and Hopkinson, 1976; Aebersold et al., 1987), and *Sebastes*-specific procedures (Seeb, 1986). Enzyme screening was designed to detect interspecific allelic mobility differences and to identify intraspecific multilocus enzymes by tissue. Therefore, each tissue type from both rougheye and shortraker rockfish were run together on each gel buffer. Of 47 enzymes screened, 23 enzymes representing 29 loci were resolved for all rougheye rockfish except the Russian collection, for which 25 loci were resolved, and the collections from regions 9b, 14a, and 16a, for which only liver samples were taken and 7 loci were resolved (data available from senior author). Twenty-nine loci were resolved for all shortraker rockfish collections except the Russian collection, for which 24 loci were resolved (data available from senior author). The loci used in subsequent analyses and the level of variation are listed in Table 2. Nomenclature for identified loci were assigned according to the American Fisheries Society guidelines for standardization (Shaklee et al., 1990).

Data analysis

Fish sampled from stations in close proximity were combined to form regional collections (Table 1 and Fig. 1). The software package GENEPOP (vers. 3.4, Montpellier University, Montpellier, France) was used to calculate genotypic frequencies for each region and to test for departure from expected Hardy-Weinberg equilibrium frequencies. Homogeneity of allele frequencies among regional collections was tested with log-likelihood ratio analysis (G-test; Sokal and Rohlf, 1981). F_{is} and F_{st} were calculated with FSTAT (Goudet, 1995).

Heterogeneity among the collections and within some of the collections of rougheye rockfish was such that the fish were easily divided into two distinct "types" according to their genotypes at five loci: *ACP**, *IDDH**, *MPI**, *PGM-2**, and *XO** (Table 3 and data available from

[2] Hawkins, S. L., J. Heifetz, J. Pohl, and R. Wilmot. 1997. Unpubl. data. Genetic population structure of rougheye rockfish (*Sebastes aleutianus*) inferred from allozyme variation. Alaska Fisheries Science Center, Quarterly Report Feature, July–Aug.–Sept. Auke Bay Laboratory, 11305 Glacier Hwy., Juneau, AK 99801.

Table 1

Regional group, location, sample size (*n*) of *S. aleutianus*, *S.* sp. cf. *aleutianus*, U = unknown type of *S. aleutianus*, *S. borealis*, and latitude, longitude, depth, and date of collections.

Region	Location	*Sebastes aleutianus* (*n*)	*Sebastes aleutianus* sp.cf. (*n*)	U (*n*)	*Sebastes borealis* (*n*)	Lat. N	Long. W	Depth (*m*)	Date
1	North Washington State	79	3			47.6	125.2	118–421	1998
2	S.E. Alaska, Dixon entrance	20	21		36	54.5	133.5	152–228	1993
3	S.E. Alaska, S. Baranoff Is.	32	16		10	56.0	135.2	176–260	1993
4	S.E. Alaska, Cross Sound	27	4		15	58.1	136.9	77–249	1993
5	S.E. Alaska, Cape Fairweather	19	1	1	38	58.4	139.3	123–241	1993
5a	S.E. Alaska, Cape Fairweather	2	6	2		58.4	140.4	300–400	2001
6	S.E. Alaska, Yakutat	50	3	4	53	59.3	141.2	102–198	1993
6a	S.E. Alaska, Yakutat	6	10	6		59.2	141.1	300–400	2001
7	S.E. Alaska, Cape Suckling	22	0	1	32	59.8	143.4	81–217	1993
7a	S.E. Alaska, Cape Suckling	5	42	2		59.3	143.1	300–600	2001
8	S. of Prince William Sound	4	43			58.2	148.4	300–600	1997
8a	S. E. of Prince William Sound	2	17			59.1	147.2	300–400	2001
9	Kodiak Island, S.W.	12	86		99	56.3	152.1	270–400	1996
9a	Kodiak Island, S.W.	11	0	8		58.0	152.2	140–150	2001
9b	Shumagin Island, S.W.	5	0	2		55.4	159.4	145	2001
10	South of Amlia Island								
	S. between Atka & Amlia Is	0	76		105	51.8	173.9	303–320	1994
	South of Amlia Island	0	12			51.5	173.3	163–650	1996
11	South Atka Pass								
	South Atka Pass	0	22		34	51.7	175.5	309–407	1994
	South of Atka Island	0	25	1	20	51.5	175.1	185–820	1996
12	South Tanaga Island								
	South of Tanaga Island	0	70		57	51.6	177.6	372–381	1994
	West of Tanaga Island	0	24		12	51.4	178.1	90–705	1996
13	North of Semisopochnoi Island	0	28			52.5	180.0	213	1994
14	North Atka Pass	0	30		37	52.1	175.0	108–940	1996
14a	North of Amlia Island	0	11	2		52.5	173.5	230–350	2001
15	N. of Islands of Four Mountains	0	34		12	53.0	170.1	172–630	1996
16	North Unalaska Island								
	North of Unalaska Island	5	0			53.7	167.0	195	1994
	North of Unalaska Island	6	44			53.7	167.0	121–350	1997
16a	North of Unalaska Island	8	3	1		53.6	167.5	85–303	2001
17	northwest Bering Sea, Russia	0	55			60.5	179.3E	390–384	1997
	northwest Bering Sea, Russia				55	60.3	171.4E	457–533	1997

senior author). We identified these types as *Sebastes aleutianus* and *Sebastes* sp. cf. *aleutianus* (a species that has putatively not been described but is similar to *S. aleutianus*). The *S. aleutianus* type is characterized by individuals with genomes of predominately *ACP* *100; *IDDH**100, and *500; *MPI**129; *PGM-2**100, *83, *91, and *117; and *XO**100. The *Sebastes* sp. cf. *aleutianus* type is characterized by individuals with genomes of predominately *ACP**46; *IDDH**500 and *750; *MPI* *100; *PGM-2* *83, *74, and *63; and *XO** 109. We used 25 loci to perform multidimensional scaling analysis of individual rougheye genotypes to illustrate separation of the two types.

We chose STRUCTURE, a Bayesian clustering model (Pritchard et al., 2000) to gain greater statistical rigor in identifying individual types and possible hybrids of rougheye rockfish. This model seeks to identify populations in a mixture without the availability of baseline samples from the separate populations. The proportions of each individual's genome belonging to the population identified by the model and the separate population allele frequencies are simultaneously estimated. A 90% probability interval is computed for each individual's inferred genome source proportions. For this analysis, we used 25 loci, 100,000 iterations, and a 10,000 burn-in period. This model assumes that loci are in Hardy-

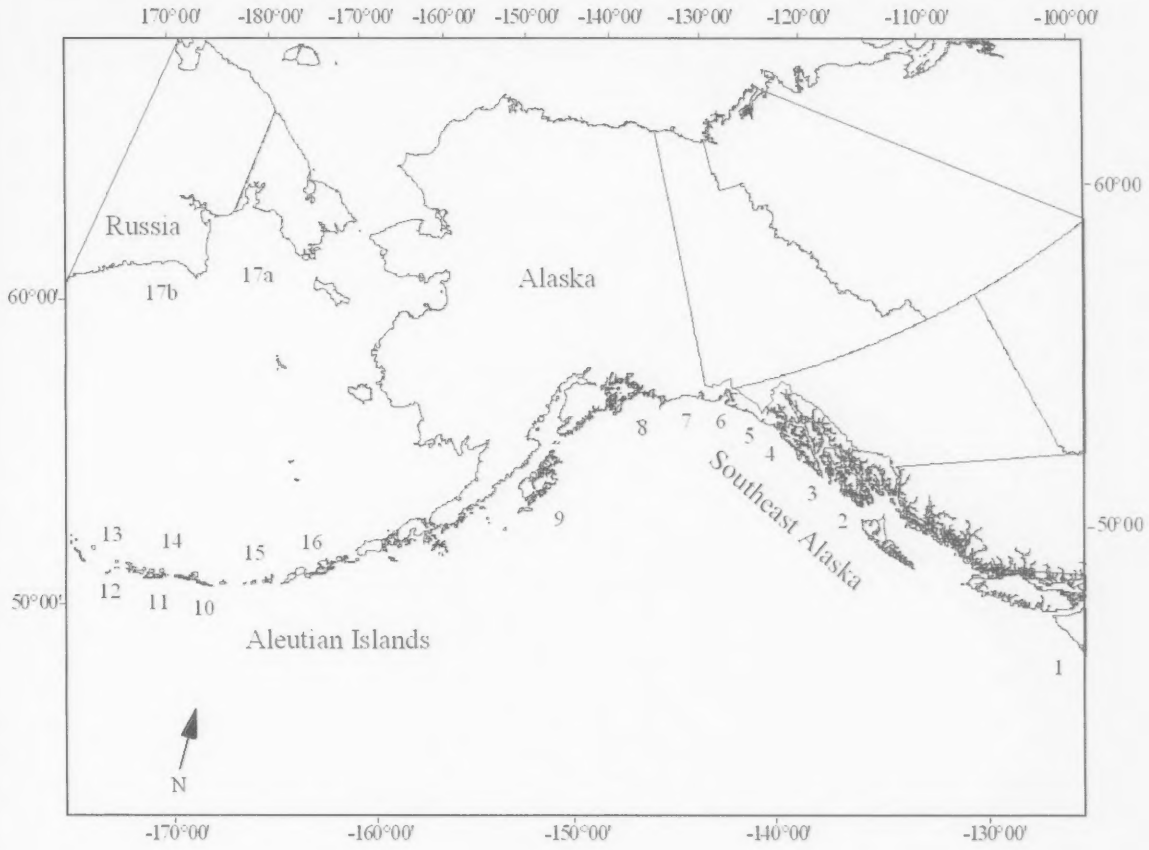

Figure 1

Location of rougheye (*Sebastes aleutianus*) and shortraker rockfish (*Sebastes borealis*) collection sites, which correspond to locations in Table 1.

Weinberg equilibrium within populations and in linkage equilibrium with one another within populations. These assumptions were tested with the PC program GENEPOP (vers. 3.4, Univ. Montpellier, Montpellier, France).

Regional groups were separated into two groups of rougheye rockfish types according to the multidimensional scaling analysis and Bayesian clustering model and were retested for Hardy-Weinberg equilibrium and homogeneity of allelic frequencies (G-test) among regions for each type. Chord distance (Cavalli-Sforza and Edwards, 1967) for 25 loci was used to assess the overall similarities of allelic frequencies for the two rougheye rockfish types with multidimensional scaling analysis (Rohlf, 2000). Only 25 loci were used because the Russian collection was missing data at 4 loci. Regions 9b, 14a, and 16a were therefore not included in these analyses because of the limited number of loci available.

Because the two rougheye rockfish types exhibited a distinct yet puzzling pattern of distribution—nearly all *S.* sp. cf. *aleutianus* in the Aleutian Islands, nearly all *S. aleutianus* in the central Gulf of Alaska, and both types in sympatry in Southeast Alaska—we collected rougheye rockfish at different depths in 2001 (regions 5a–9a, 9b, 16a). We ran a Mann-Whitney rank sum test (SigmaStat, vers. 2.0, SPSS, Chicago, IL) to test for significant differences of the mean, standard deviation, and range of depths between the two rougheye rockfish types. A single depth of 350 m was used to approximate depth of catch for the 2001 Southeast Alaska rougheye rockfish collections (regions 5a, 6a, and 7a) because depths were reported only as a range from 300 to 600 m. Had we chosen a deeper average depth in the range, the difference in depth between the two rougheye rockfish types would have been (and in actuality may be) even greater. Because the two rougheye types were found in sympatry, we analyzed the length data to determine if size differences existed between the two types. Linear regressions were used to examine the relationships between length (tip of snout to fork of tail) and depth of capture of both shortraker rockfish and the two rougheye rockfish types.

Table 2

Enzymes with associated International Union of Biochemistry Numbers (IUBNC), locus name (Shaklee et al., 1990), tissue(s), buffer(s), and level of variability for *Sebastes aleutianus*. RE=both *Sebastes* sp. cf. *aleutianus* and *Sebastes aleutianus*, and SR=*Sebastes borealis*. Tissue: M=muscle; H=heart; and L=liver. Buffers: 1= R (Ridgway et al., 1970); 2 = MF (Markert and Faulhaber, 1965); 3=CA6.1 and 4- CA6.9 (Clayton and Tretiak, 1972, modified pH); 5 = TC (Shaw and Prasad, 1970); and 6 = CAME7.4 (modified from Clayton and Tretiak, 1972). Var. RE and Var. SR: 0 = monomorphic; 1 = frequency common allele >0.95; 2 = frequency common allele <0.95 for at least one region. — = Loci were not reliably scored in that species. + = loci were not reliably scored in all populations and were not used in most analyses.

Enzyme	IUBNC no.	Locus	Tissue	Buffer	Var. RE	Var. SR
Acid phosphatase	3.1.3.2	*ACP**	L	3	2	—
Aconitate hydratase	4.2.1.3	*mAH**	H	5,6	1	1
		*sAH**	L	3,4	2	1
Adenosine deaminase	3.5.4.4	*ADA-1**	M,H	3,6	0	2
Adenylate kinase	2.7.4.3	*AK**	M,H,L	6	0	0
Alcohol dehydrogenase	1.1.1.1	*ADH**	L	3	2[1]	2
Aspartate aminotransferase	2.6.1.1	*sAAT**	L	1	2[1]	0
		*mAAT**	M,H,L	3,4,6	1	1
beta-N-Acetylgalactosaminidase	3.2.1.53	*bGALA**	L	4	0[1]	0
Creatine kinase	2.7.3.2	*CK-1**+	H	3,6	1	0
Fumarate hydratase	4.2.1.2	*FH**	H,L	5	0[1]	1
Glucose-6-phosphate isomerase	5.3.1.9	*GPI-A**	M,H,L	1,3	1	2
		*GPI-B**	M,H	1,3	1	1
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3PDH**	M	2	0	1
Iditol dehydrogenase	1.1.1.15	*IDDH**	L	1	2	—
Isocitrate dehydrogenase	1.1.1.42	*IDHP-1**+	H	3	1	1
		*IDHP-2**	L	3	1	1
Lactate dehydrogenase	1.1.1.27	*LDH**	M,H	3	0	0
Malate dehydrogenase	1.1.1.37	*MDH-1**	M,H	3,6	1	—
		*MDH-2**	M,H,L	3,4,6	1	1
Malic enzyme	1.1.1.40	*mMEP**	M,H	3,6	2[1]	2
Mannose-6-phosphate isomerase	5.3.1.8	*MPI**	H	6	2	2
Dipeptidase (glycyl-leucine)	3.4.-.-	*PEPA**	M,H,L	2	2	1
Tripeptide aminopeptidase (leu-gly-gly)	3.4.-.-	*PEPB**	M,H,L	1	0	0
		*PEPD**+	M,H	2	—	2
		*PEP-LT**+	M,H	2	—	1
Phosphoglucomutase	5.4.2.2	*PGM-1**	M,H,L	1,5	2[1]	2
		*PGM-2**	H	5	2	2
6-Phosphogluconate dehydrogenase	1.1.1.44	*PGDH**	M,H,L	3	2[1]	0
Triose-phosphate isomerse	5.3.1.1	*TPI-1**	M,H	1,3	0	0
		*TPI-2**	M,H	1,3	—	2
Xanthine Oxidase		*XO**	L	2	2[1]	0

[1] *Sebastes* sp. cf. *aleutianus* level of variablity was 1.

Parasite analysis

Although not an objective of the study, parasites were opportunistically sampled from the 2001 Gulf of Alaska collections of rougheye rockfish to determine if depth or species subtype might have been a factor in the geographical segregation noted by Moles et al. in 1998. This would also allow us to determine if the parasite data supported results of the current allozyme work. The rougheye rockfish were examined for the proportion of fish with the gill parasites *Neobrachiella robusta*, *Trochopus trituba*, or the visceral parasite *Corynosoma* sp. by using the procedures of Moles et. al. (1998). A categorical analysis of variance (SAS procedure, CATMOD: vers. 8.02. Cary, NC 1989) was used to test whether parasite prevalence differed among the two types of rougheye rockfish.

Table 3

Allelic frequencies of five loci for all samples by type that best distinguish *Sebastes aleutianus* and *Sebastes* sp. cf. *aleutianus*.

Locus	*n*	Allele					
*ACP**		100	46	83			
aleutianus	242	0.896	0.087	0.017			
sp. cf. *aleutianus*	486	0.094	0.905	0.001			
*IDDH**		100	500	750	999		
aleutianus	287	0.73	0.268	0.002	0		
sp. cf. *aleutianus*	658	0.03	0.507	0.462	0.001		
*MPI**		100	129	110			
aleutianus	283	0.343	0.656	0.001			
sp. cf. *aleutianus*	540	0.74	0.26	0			
*PGM-2**		100	83	74	63/69/59**	80	91/117**
aleutianus	270	0.775	0.185	0.005	0	0.002	0.028
sp. cf. *aleutianus*	586	0.003	0.333	0.508	0.147	0.009	0
*XO**		100	109				
aleutianus	295	0.844	0.156				
sp. cf. *aleutianus*	660	0.011	0.989				

** indicates pooled alleles.

Results

Shortraker rockfish and rougheye rockfish had different common alleles (fixed) for 10 of 29 loci examined (*sAH**, *CK-A1**, *GPI-A**, *G3PDH**, *IDHP-2**, *PEPA**, *PEPB**, *PEP-LT**, *PGM-2**, and *SOD**). These are inexpensive markers that can be used to differentiate shortraker rockfish from rougheye rockfish when precise field identification, particularly in younger fish, is necessary but difficult.

Shortraker rockfish

Nine loci (31%) were monomorphic for all regions, 11 loci (38%) were variable (with the frequency of the common allele greater than 0.95 for all regional groups), and 9 loci (31%) had a common allele frequency of less than 0.95 for at least one regional group. For the Russian collection, data were unavailable from five loci (*FH**, *mIDHP**, *MPI**, *PGM-2**, and *TPI-2**). Average heterozygosity of each regional group fell within a narrow range of 0.09–0.11, and produced an overall average for 29 loci of 0.10. All regional genotypic proportions closely agreed with those expected under Hardy-Weinberg equilibrium; of 128 chi-square tests, only four (3%) differed significantly ($P<0.05$) from expected values. No significant ($P<0.05$) heterogeneity was detected with G-tests among regional groups, and thus no subpopulations or stock structure was evident with this suite of allozymes.

Although no genetic differentiation was detected among shortraker rockfish throughout their geographic distribution, size of fish and depth of capture differed between shortraker rockfish from the Aleutian Islands and those from Southeast Alaska. Aleutian Island shortraker rockfish were significantly smaller (mean 43.6 cm [±SD 7.0], range: 24–70 cm) and were caught in deeper water (309–407 m) than Southeast Alaska shortraker rockfish (mean 66.5 cm [±SD 10.5], range: 45–101 cm at 138–260 m depths). A regression of fish length on depth of capture yielded a significant r^2 value of 0.452 ($P<0.001$).

Rougheye rockfish

Significant departure from Hardy-Weinberg equilibrium occurred in 37 out of 226 possible tests (16%); a value greater than the 11 that would be expected by chance alone at the $P=0.05$ level of probability (Table 4). Thirty-six of the departures were due to an absence of heterozygotes, a situation known as the Wahlund effect, which typically indicates the presence of a mixture of populations for presumably neutral genetic loci. Most of the departure from Hardy-Weinberg expectations occurred at *ACP**, *IDDH**, *MPI**, *PGM-2**, and *XO**. Only

Table 4

Loci not in Hardy Weinberg equilibrium ($P<0.05$). N/A = insufficient sample size for analysis.

Location	Mixture	*S. aleutianus*[1]	*S.* sp. cf. *aleutianus*[1]
Dixon Entrance	*ACP, sAH, IDDH PGM-2, XO*	*ACP, IDDH*	*ACP, IDDH*
S. Baranof Island	*ACP, IDDH, XO PGM-1, PGM-2*	*ACP, PGM-1*	None
Cross Sound	*ACP, IDDH, XO*	None	N/A
Cape Fairweather	*ACP, PGM-2*	*ACP*	N/A
Yakutat	*ACP, MPI PGM-2, XO*	*MPI*	N/A
Cape Suckling	None	None	N/A
Prince William Sound	*IDDH, PGM-2, XO*	N/A	None
Kodiak	*ACP, IDDH PGM-2, XO*	None	*ACP*
Amlia Island	None	N/A	None
South Atka Pass	*MPI*	N/A	*MPI*
South Tanaga Island	*PEPA, PGM-2*	N/A	*PEPA*
North Semisopochnoi Island	*IDDH*	N/A	*IDDH*
North Atka Pass	None	N/A	None
N. Is. Of Four Mountains	None	N/A	None
North Unalaska Island	*ACP, PGM-2, XO*	*ACP, XO*	None
Washington	*sAH, IDDH, MPI PGM-2*	*sAH, MPI*	N/A
Russia	None	N/A	None

[1] As determined from the program STRUCTURE (Pritchard et al., 2000).

*PGM-2** in the South Tanaga Island sample was due to an excess of heterozygotes.

Inbreeding coefficients (F_{is}) indicated deviation from panmixia. The values ranged from −0.050 for *IDHP-1** to 0.772 for *ACP**. The mean value over all loci in all collections was 0.140. Statistically significant F_{is} values were found at *ACP** (0.521), *MPI** (0.135), *PGM-2** (0.109), and *XO** (0.524). All were the result of heterozygote deficiencies. The mean F_{is} value for the *S. aleutianus* type collections dropped to 0.062 and for the *S.* sp. cf. *aleutianus* types, to 0.048.

Eight of the loci showed statistically significant F_{st} values: *sAAT** (0.007), *ACP** (0.572), *sAH** (0.037), *IDDH** (0.189), *MDH-2** (0.007), *MPI** (0.123), *PGM-2** (0.206), and *XO** (0.551). The mean F_{st} value for all loci in all collections was 0.215. When analyzed by pure *S. aleutianus*-type and *S.* sp. cf. *aleutianus*-type, the mean F_{st} values dropped to 0.013 and 0.012, respectively.

Two rougheye types

The results of the rougheye rockfish analyses allowed us to segregate rougheye rockfish individuals into two types: *S. aleutianus* and *Sebastes* sp. cf. *aleutianus*. Multidimensional scaling analysis with individual genotypes (Fig. 2) yielded two distinct clusters with little overlap. This outcome was confirmed by the Bayesian clustering model in STRUCTURE (Pritchard et al., 2000), which identified two types. We calculated the inferred source proportions of genomes for 1027 individuals using 25 loci that were scored in most individuals. One hundred sixty-six individuals were missing data for more than 30% of the 25 loci used and were omitted from the analysis. Most of the individuals had a very high proportion of their genome from one type; for 851 individuals, the program assigned at least 0.80 of the individual's genes to one of the two ancestral lines, and all had an upper 90% probability limit that included 1.0. These fish were likely all purebreds. Ten individuals had an inferred proportion of ancestry from one lineage of less than 0.80 and two did not include an upper probability interval of 1.0. These individuals were possibly hybrids. If any of these 10 individuals were actual hybrids of the two rougheye rockfish types, none were of the first generation (i.e., heterozygotes at all differentiating loci).

Significant differences of allele frequencies (G-test) were detected between the two types at 14 loci: $P<0.001$ for *sAAT**, *ACP**, *ADH**, *sAH**, *IDDH**, *MDH-2**, *mMEP**, *MPI**, *PGDH**, *PGM-1**, *PGM-2**, and *XO** ; and $P<0.05$ for *mAAT** and *GPI-B**. When the two types were analyzed independently by area (Table 4), all but two collections were in Hardy-Weinberg equilibrium

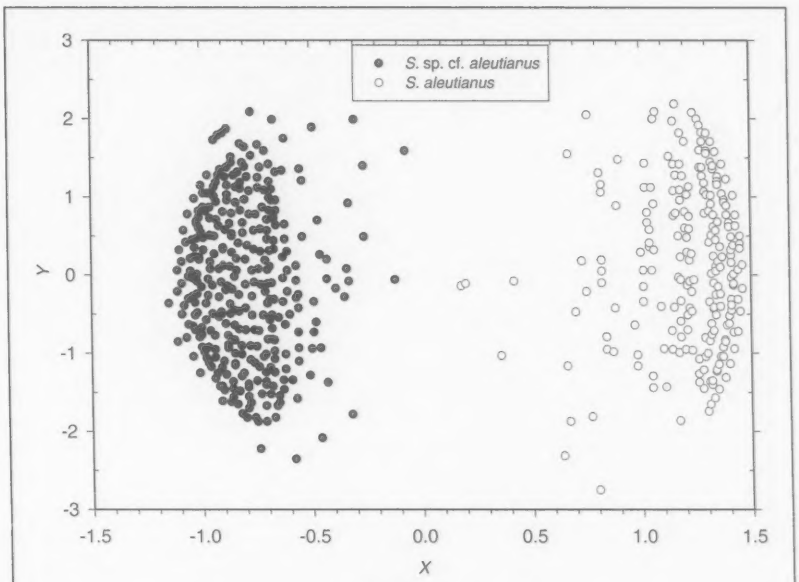

Figure 2

Multidimensional scaling analysis of individual rougheye rockfish (*Sebastes aleutianus*) genotypes for 25 loci.

(South Tanaga Island *Sebastes* sp. cf. *aleutianus* type, P=0.042, and North Unalaska Island *S. aleutianus* type, P=0.023). The G-test analysis indicated no heterogeneity among regions except for the Russian sample, which was significantly different from all other samples (P<0.05). Average heterozygosity was 0.09 for *S. aleutianus* and 0.08 for *Sebastes* sp. cf. *aleutianus*.

A significant difference in overall depth of capture (P<0.001) was detected between *Sebastes* sp. cf. *aleutianus* (mean 330+ m) and *S. aleutianus* (mean 208 m). We obtained both shallow and deep collections from the central Gulf of Alaska. The fish captured at shallow depths, 77–249 m (regions 4–7, 9a, 9b, n=134), were nearly all (94%) *S. aleutianus*, whereas the deeper dwelling fish, 270–600 m (regions 5a–7a, 8, 8a, 9, n=204), were mostly (87%) *Sebastes* sp. cf. *aleutianus*. Both types were captured, some within a single haul, in southern Southeast Alaska (regions 2 and 3, n=89) at depths of 150–260 m (Fig. 3).

A highly significant correlation of fish length (15–65 cm) and depth of capture (77–260 m) was detected for *S. aleutianus* in Southeast Alaska, with smaller fish in shallower water and larger fish in deeper water (r^2=0.415, P<0.001). No length-depth trend was noted for *Sebastes* sp. cf. *aleutianus*.

Results of the parasite analysis for the 2001 rougheye rockfish showed that *Sebastes* sp. cf. *aleutianus* had a significantly higher prevalence of both *Neobrachiella robusta* (P=0.003) and *Trochopus trituba* (P=0.022) than did *S. aleutianus* (Table 5).

Discussion

The most notable conclusion of our study was that two genetically distinct types of rougheye rockfish exist. This conclusion corroborates prior biochemical studies in which Tsuyuki et al. (1968) and Tsuyuki and Westrheim (1970) conducted hemoglobin electropherogram analyses on *S. aleutianus* and *S. caenaematicus* (=*S. borealis*) and detected four blood types. Three blood types characterized *S. aleutianus*—two distinct types and a rare hybrid type. The fourth type characterized *S. borealis*. Seeb (1986) examined allozymes from several species of North Pacific rockfish and found two color morphs of rougheye rockfish fixed for alternate alleles at three loci. At one of the loci, *ACP**, we detected a small percentage of a shared allele, likely because of our larger sample size. We were unable to resolve the other two loci, *GAP** (IUBNC no. 1.2.1.12 Glyceraldehyde-3-phosphate dehydrogenase,) and *GAM** (B-Galactosaminidase). Although we are unable to report fixed loci differences between the two rougheye rockfish types, we did detect significant allele frequency differences at nearly half of the loci examined. Allelic mobilities of *Sebastes aleutianus* were similar to those of Seeb's *"Sebastes aleutianus,"* and allelic mobilities of *Sebastes* sp. cf. *aleutianus* were similar to Seeb's *"Sebastes aleutianus* unknown." Because simultaneous hemoglobin and allozyme studies have never been done, we are currently unable to correlate allozyme types with the blood types reported by Tsuyuki et al. (1968) and Tsuyuki and Westrheim (1970).

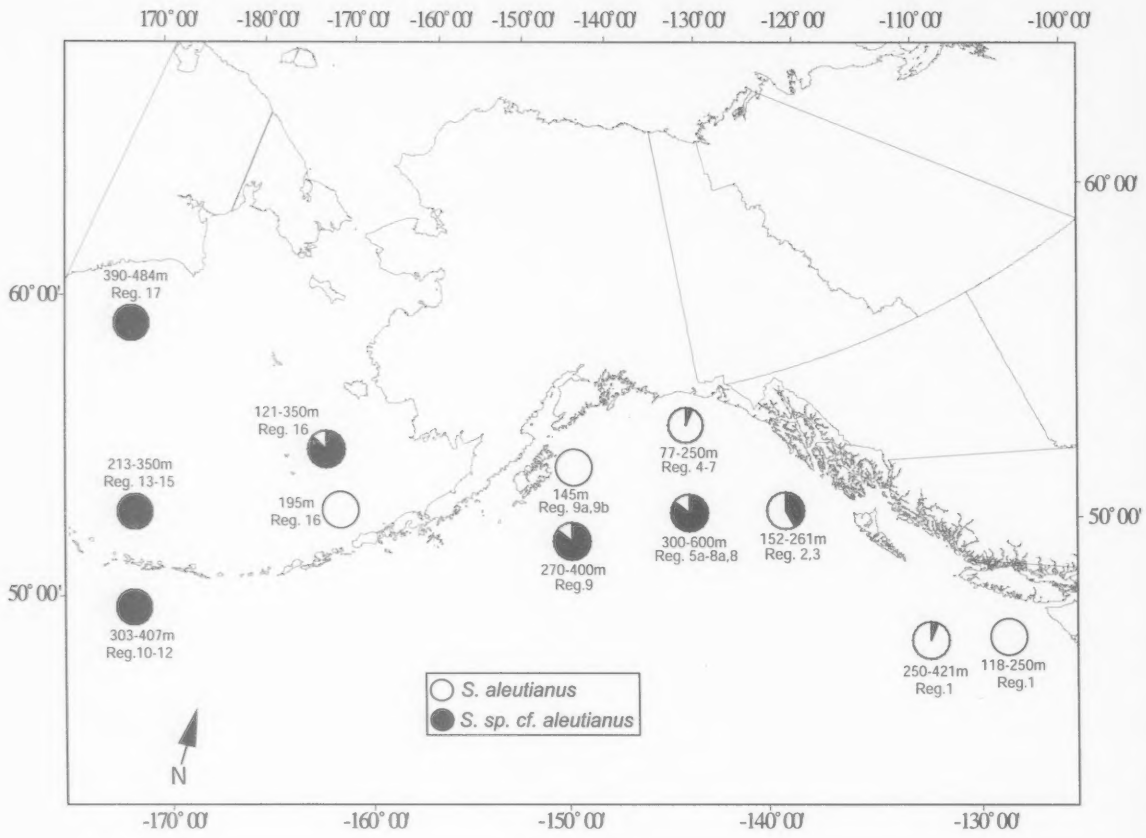

Figure 3

Proportions of *Sebastes aleutianus* and *S.* sp. cf. *aleutianus* in relation to depth of capture.

Table 5

Prevalence of parasites (percentage) in both rougheye rockfish types and results of categorical analysis of variance.

	Parasite prevalence		Significance probability	
	sp. cf. *aleutianus* (n=61)	*S. aleutianus* (n=18)	Type	Size of fish
Neobrachiella robusta	0.57	0.11	0.003*	0.442
Trochopus trituba	0.49	0.17	0.022*	0.339
Corynosoma sp.	0.90	0.83	0.448	0.326

The Cavalli-Sforza-Edwards (CSE) chord distance (for 29 loci) between the two *S. aleutianus* types, 0.35 (SD=0.05), was a value comparable to that for other closely related rockfish species. Seeb (1986) reported CSE distances between rockfish species ranging from 0.07 to 0.75 for 28 loci. Identical mobilities at the majority of loci indicated a close relationship between the two types, which probably existed as a single type at an earlier geologic time. Given that Tsuyuki and Westrheim (1970) detected (2%) hybrids of the two blood types and we did not detect fixed differences between the two rougheye rockfish types, some gene flow may be occurring. However, the low effective number of migrants and the sympatric distribution of the population indicate that the gene flow is limited. Because rockfish have internal fertilization, sibling species may co-occur and there is little chance of cross-fertilization of gametes.

The initial objective of our study did not include collection of morphological data, but in light of the genetic differences detected, and the color morphs detected by Seeb (1986), morphology of the two lineages should be more closely examined. Upon processing the 2001 fish, we noted that many were easily identified as either light or dark in color, although some appeared intermediate. The obvious light-colored individuals were all found genetically to be *S. aleutianus*, whereas the darker specimens were typically *Sebastes* sp. cf. *aleutianus*. Although Tsuyuki and Westrheim (1970) reported no distinguishing meristic or morphometric characters between *S. aleutianus* blood types, Seeb (1986) separated rougheye rockfish morphologically and by color into two groups: one that was light pink and had spines under the orbit of the eye (*S. aleutianus*); the other was darker and had a considerable area of black on the mouth and jaw and often lacked orbital spines (*S. aleutianus* unknown). The lack of orbital spines in *Sebastes* sp. cf. *aleutianus* is an important observation because this feature is a key characteristic in distinguishing *S. aleutianus* from *S. borealis*.

Initial observations of the distribution of the 1993 rougheye samples displayed a pattern of predominately *S. aleutianus* in the Gulf of Alaska and almost entirely *S.* sp. cf. *aleutianus* in the Aleutian Islands. A parasite study (Moles et al., 1998) performed on the same rougheye rockfish reported a significantly greater ($P<0.05$) prevalence of three parasites in the Aleutian Island samples. Upon close examination of the depth of the sample collection, we noted that the Aleutian Islands samples were collected in deeper waters than those collected in the Gulf of Alaska. Thus subsequent sampling strategies focused on a possible depth niche. Parasite data from both the shallow and deep water 2001 rougheye collections showed a significant prevalence of two parasites in the deeper host, *Sebastes* sp. cf. *aleutianus* (Table 5). The prevalence of the parasite *T. trituba* may be dependent on host habitat. Because the hosts (two *aleutianus* types) exhibited significantly different prevalences of the parasite *T. tributa*, they may be using different resources (different diets) and (or) exhibiting ecological segregation (Moles et al., 1998).

Both *Sebastes aleutianus* types are found in the Gulf of Alaska and occur in sympatry, although the majority of *S.* sp. cf. *aleutianus* are distributed at deeper depths (Fig. 3). Bathymetric segregation has been noted in other closely related rockfish. *Sebastes fasciatus* and *Sebastes mentella* in the Atlantic Ocean elude abundance estimates because of a lack of easily identifiable morphological characteristics. In the laboratory, these two species are nearly fixed at alternate alleles at the allozyme locus MDH*. A bathymetric segregation is detected, where *S. fasciatus* is found at depths of 132–315 m, *S. mentella* at depths greater than 355 m, and both species and possible hybrids at intermediate depths (Rubec et al., 1991). Another species pair, *Sebastes carnatus* and *Sebastes chrysomelas*, are both shallow-dwelling species (depth less than 20 m) that coexist only in a narrow zone of overlap that separates exclusive depth ranges. Factors organizing their segregation are largely behavioral because both species expand their depths in the absence of the other species (Larson, 1980). Rougheye rockfish may exhibit a similar strategy. Deep sampling efforts near Washington state have indicated that the distribution of *S.* sp. cf. *aleutianus* may diminish in southern ranges and it appears that the distribution of *S. aleutianus* does not extend to the western Aleutian Islands and Asia. This pattern of distribution has been noted in other closely related species, such as the northern and southern species of *Lepidopsetta* (Orr and Matarese, 2000). The poorly understood ontogenetic and seasonal movements of rougheye and other rockfish further confound the picture. For example, *S. altivelis* and *S. alascanus* were long thought to be deep-dwelling and shallow-dwelling congeneric species, respectively. It is now known that *S. altivelis* is a permanent deepwater resident, whereas *S. alascanus* settles in shallow water, then migrates to deep water with the onset of sexual maturity. Competition between these two species may be reduced by size differences; for example, where they are sympatric, *S. alascanus* is much larger than *S. altivelis* (Vetter and Lynn, 1997). Although length data for rougheye rockfish in sympatry are limited, a single haul ($n=29$) near Dixon Entrance (depth of capture= 213 m) yielded both types, and nearly all the *S. aleutianus* individuals were much larger (mean 56.3 cm) than *Sebastes* sp. cf. *aleutianus* (mean 37.2 cm). No age data are currently available to add insight into these results. The trend of smaller fish in shallower waters and larger fish in deeper waters was significant for *S. aleutianus* in the Gulf of Alaska. This was not detected for *Sebastes* sp. cf. *aleutianus*, although their full depth range may not have been sampled. Perhaps only younger *Sebastes* sp. cf. *aleutianus* were collected, and the older, larger fish are deeper and remain unsampled. It would be beneficial to analyze juvenile rougheye rockfish to ascertain genetic type composition at different depths.

A significant size difference existed between fish in the Gulf of Alaska and those of the Aleutian Islands, especially among shortraker rockfish. Aleutian fish were smaller, despite collection at greater depth. Growth and age of maturation differences among differing latitudes and longitudes have been noted in other rockfish species (Westrheim, 1973; Archibald et al., 1981; Field, 1984; Lunsford, 1999). The size difference among shortraker rockfish has been previously noted (Orlov, 2001; Matala, 2004) and raises more questions than it answers. It is still unknown whether these differences are caused by different age classes or regional ecological differences.

Allozyme data did not reveal heterogeneity within either rougheye rockfish type or within shortraker rockfish throughout the sampled geographic range. Although no heterogeneity was detected with our suite of allozyme loci, other genetic markers, such as microsatellite loci, may provide finer resolution of population structure. A recent study of shortraker microsatellite variation revealed geographically restricted homogeneity among allele frequencies—a model consistent with the assump-

tion of limited movement (Matala, 2004). Conversely, Orlov (2001) proposed a synopsis of horizontal adult migration (with increased size of shortraker rockfish at spawning grounds) and oceanic dispersal of larvae and juveniles.

In conclusion, it appears there are species-level differences between the two rougheye rockfish types. We have considered the darker morph *Sebastes* sp. cf. *aleutianus* as the new type. The paler *S. aleutianus* morph conforms more to the original *S. aleutianus* type description, an individual of which was captured at a 55-m depth in the Gulf of Alaska. It is likely that the distribution of the new species *S.* sp cf *aleutianus* stretches from the Gulf of Alaska and west to Asia. The distribution of *S. aleutianus* encompasses the Gulf of Alaska and extends south to California, and the species is found in more shallow waters where it is sympatric with *S.* sp cf *aleutianus*. An understanding of the basic life history, distribution, and biomass of a species is critical for successful resource management. Ito (1999) suggested that the major fisheries management survey effort is the NMFS Gulf of Alaska triennial trawl survey, which may be inadequate to assess the shortraker-rougheye rockfish assemblage because its multispecies sampling design covers mostly depths less than 200 m. This survey may, therefore, be completely missing *Sebastes* sp. cf. *aleutianus* altogether. An important consideration for management is knowledge of exploitation rates. Given the sensitivity of long-lived rockfish species to over-exploitation, basic biological studies should be undertaken of these species to understand characteristics such as growth, maturity, and natural mortality.

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The reproductive cycle of the thorny skate (*Amblyraja radiata*) in the western Gulf of Maine

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Abstract—The thorny skate (*Amblyraja radiata*) is a large species of skate that is endemic to the waters of the western north Atlantic in the Gulf of Maine. Because the biomass of thorny skates has recently declined below threshold levels mandated by the Sustainable Fisheries Act, commercial harvests from this region are prohibited. We have undertaken a comprehensive study to gain insight into the life history of this skate. The present study describes and characterizes the reproductive cycle of female and male thorny skates, based on monthly samples taken off the coast of New Hampshire, from May 2001 to May 2003. Gonadosomatic index (GSI), shell gland weight, follicle size, and egg case formation, were assessed for 48 female skates. In general, these reproductive parameters remained relatively constant throughout most of the year. However, transient but significant increases in shell gland weight and GSI were observed during certain months. Within the cohort of specimens sampled monthly throughout the year, a subset of females always had large preovulatory follicles present in their ovaries. With the exception of June and September specimens, egg cases undergoing various stages of development were observed in the uteri of specimens captured during all other months of the year. For males (n=48), histological stages III through VI (SIII–SVI) of spermatogenesis, GSI, and hepatosomatic index (HSI) were examined. Although there appeared to be monthly fluctuations in spermatogenesis, GSI, and HSI, no significant differences were found. The production and maintenance of mature spermatocysts (SVI) within the testes was observed throughout the year. These findings collectively indicate that the thorny skate is reproductively active year round.

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The thorny skate (*Amblyraja radiata*) is a member of the family Rajidae (Robins and Ray, 1986; Collette and Klein-MacPhee, 2002). It is a cosmopolitan species, endemic to both sides of the Atlantic Ocean, from Greenland and Iceland to the English Channel in the eastern Atlantic (Compagno et al., 1989), and from Greenland and Hudson Bay, Canada, to South Carolina, in the western Atlantic (Robins and Ray, 1986; Collette and Klein-MacPhee, 2002). Despite such a wide distribution, knowledge pertaining to the reproductive biology of this species is limited. Templeman (1982) reported the occurrence of egg capsules in *A. radiata*, and Templeman (1987), Del Río (2002), and Sosebee[1] examined size at sexual maturity.

In the Gulf of Maine, these skates were generally discarded as bycatch because of their low commercial value NEFMC.[2,3] Recently, the rapidly expanding markets for skate wing has made this species commercially more viable, especially because *A. radiata* meets the minimum 1¼ pound-cut pectoral fin size sought by processors (Sosebee[1]; NEFMC[2]). Although no comprehensive published data for reproductive cycles currently exist for thorny skates in the Gulf of Maine, information from the few skate species studied so far indicates that sexual maturity at a late age, low fecundity, and a relatively long life span may also be characteristics of *A. radiata's* life history (Winemiller and Rose, 1992; Zeiner and Wolf, 1993; Francis et al., 2001; Frisk et el., 2001; Sulikowski et al., 2003). When these characteristics are coupled with the practice of selective removal of large individuals, the thorny skate population in the Gulf of Maine may be highly susceptible to over-exploitation by commercial fisheries (Brander 1981; Hoenig and Gruber, 1990; Casey and Myers 1998; Dulvy et al., 2000; Frisk et al., 2001). Because of an in-

[1] Sosebee, K. 2002. Maturity of skates in northeast United States waters. Scientific Council Research Document NAFO. no. 02/134, 17 p. [Available from the Northwest Atlantic Fisheries Organ., Dartmouth, NS.]

[2] New England Fishery Management Council (NEFMC). January 2001. 2000 stock assessment and fishery evaluation (SAFE) report for the northeast skate complex, 179 p. NEFMC, 50 Water Street, Mill 2 Newburyport, MA 01950.

[3] New England Fishery Management Council (NEFMC). 2003. Skate fisheries management plan, 142 p. 50 Water St., Mill 2 Newburyport, MA 01950.

creasing commercial importance, declines in biomass levels, and a paucity of specific biological information, commercial harvests of thorny skates in the U.S. portion of the western North Atlantic are now prohibited. Thus, obtaining life history information for this skate species is not only timely (Simpfendorfer, 1993; Frisk et al., 2001), but it has become imperative. The objective of the present study was to describe the patterns of several morphological reproductive parameters manifested during the reproductive cycle of female and male *A. radiata* collected in the western Gulf of Maine.

Materials and methods

Sampling

Thorny skates were captured by otter trawl in an area approximately 900 square miles centered at 42°50′N and 70°15′W in the Gulf of Maine. These locations varied from 30 to 40 km off the coast of New Hampshire. Collection of skates occurred between the 10th and 20th of each month beginning May 2001 and ending May 2003. A comparison of samples taken from the same month between different years revealed no variability. Furthermore, the skates sampled in the present study were obtained from the same population and geographic location. Thus, the data from the same months for different sampling years were grouped together.

Skates were maintained alive on board the FV *Mystique Lady* until transport to the University of New Hampshire's Coastal Marine Laboratory (CML). There, individual fish were euthanized (0.3 g/L bath of MS222). Total length (TL in mm) was measured as a straight line distance from the tip of the rostrum to the end of the tail, and disc width (DW in mm) as a straight line distance between the tips of the widest portion of pectoral fins. Total wet weight (kg) was also recorded. For males, clasper length was measured as the straight line distance from the posterior point of the cloaca to the end of the clasper. The gonadosomatic index (GSI) and hepatosomatic index (HSI) were calculated as gonad weight divided by total body weight multiplied by 100, and liver weight divided by total body weight multiplied by 100, respectively. The epigonal organ was included in both male and female GSI measurements because of its close association with the gonads (Maruska et al., 1996).

Criteria used to determine reproductively active skates

Females whose reproductive tracts contained ovarian follicles with a minimum diameter of 25 mm and had a shell gland weighing at least 30 g were considered mature (capable of egg encapsulation and oviposition). These numbers were determined from our observations of reproductive tracts containing egg cases that were either fully formed or undergoing various stages of formation. Males with calcified claspers 200 mm long or greater, and with a proportion of mature spermatocysts in the testes of 25% or greater were considered reproductively capable of fertilizing an ovulated follicle. These criteria are consistent with previous studies that reported similar characteristics for other mature elasmobranch species (Koob et al., 1986; Heupel et al., 1999; Conrath et al., 2002; Sulikowski et al., 2004). Male and female thorny skates that did not meet all the criteria were considered to be immature. We also looked for some other indicators of reproductive activity, such as mating bites on female pectoral fins, and evidence of mating activity on male claspers, but they were either absent or not apparent in specimens examined during the study. Sperm storage was not assessed in the present investigation.

Gross morphology of the female reproductive tract

After removal of reproductive tracts, the ovaries, shell glands, and uteri were dissected out, blotted dry, and weighed to the nearest gram. Ovarian follicle dynamics were evaluated by measuring the diameter (with a caliper) and counting all follicles ≥1 mm in diameter (Tsang and Callard, 1987; Snelson et al., 1988; Sulikowski et al., 2004). For this data set, we averaged the size of the largest single follicle found on the right and left ovaries of each skate. Average follicle diameters, average ovary weights, and average shell gland weights were analyzed to assess temporal patterns during the reproductive cycle.

Histology of the testis

From male specimens, testes were removed, blotted dry, and weighed to the nearest gram. A single 2–3 mm thick segment was removed from the central portion of a single lobe in the medial area of an individual testis (Maruska et al., 1996; Sulikowski et al., 2004), placed in a tissue cassette, and fixed in 10% buffered formalin until processed by the University of New Hampshire Veterinary Diagnostic Laboratory. There, the sample was dehydrated, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Prepared slides of testicular tissue were examined and classified into stages of spermatogenic development following the criteria described by Maruska et al. (1996), Hamlett and Koob (1999), and Tricas et al. (2000). For the developmental stages of spermatogenesis described in other elasmobranchs, hormone analyses have confirmed that stages III through VI are associated with reproductive readiness (Heupel et al., 1999; Tricas et al. 2000; Sulikowski et al. 2004). For this reason, we focused our efforts on these specific stages in the thorny skate. Briefly, these stages have the following characteristics: stage III, spermatocysts; stage IV, spermatids; stage V, immature spermatozoa; and stage VI, mature spermatocysts (Maruska et al., 1996). In the present study, the mean proportion of testis occupied by each of these stages was measured along a straight line distance across one representative full lobe cross section of the testis (Maruska et al., 1996; Conrath et al., 2002).

Statistical analyses

The results are presented as means ±SEM and evaluated by Kruskal Wallis analysis of variance followed by a Tukey's *post hoc* test. Statistical significance was accepted at $P<0.05$. To determine whether a relationship exists in measured morphological and histological reproductive parameters, a Pearson correlation analysis (denoted as r) was performed.

Results

The lack of a robust sample size presents a potential limitation for our study. However, over the last decade, there has been an increasingly precipitous decline in thorny skate populations in the Gulf of Maine, especially larger size specimens (NEFMC[2,3]). These declines were evident in our sampling trips, because large, mature individuals were rarely caught in most trawls. The data presented in this article are the result of 84 sampling trips that took place over the course of two years (approximately three to four trips per month). Moreover, the recent prohibition on thorny skate landings has put an end to any prospects regarding collection of additional specimens in the foreseeable future. Thus, the data set we have presented represents the best available information on the reproductive cycle for this species.

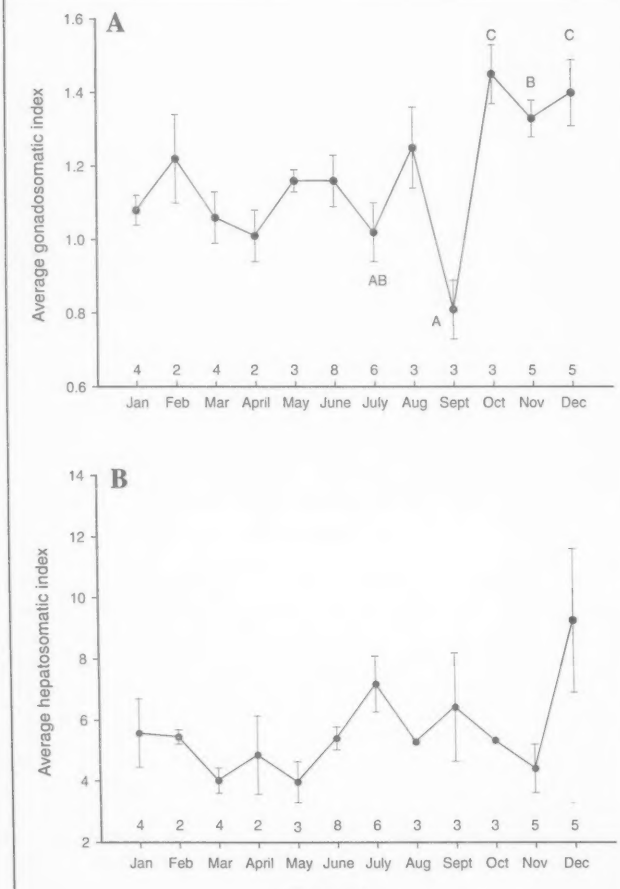

Figure 1

Monthly changes in female thorny skates (*Amblyraja radiata*): (**A**) Gonadosomatic index (GSI); (**B**) hepatosomatic index (HSI); (**C**) shell gland weight; and (**D**) diameter of the two largest follicles. Values are expressed as means ±SEM. Sample sizes are indicated above each month. Values designated with different letters are significantly different from each other ($P<0.05$).

Size ranges

Mature female skates ($n=48$) ranged from 820 to 1050 mm TL (mean=917 ±7 SEM) and from 4.4 to 10.2 kg (mean=7.7 ±0.2 SEM) in total body mass. Mature male skates ($n=48$) ranged from 800 to 1040 mm TL (mean=952 ±11 SEM), and from 5.4 to 10.8 kg (mean=8.4 ±0.3 SEM) in total body mass.

Assessment of morphological parameters in the female reproductive tract

In females, the average GSI of skates captured in July was lower ($P<0.05$) than those captured in October and December, and those from September were lower than the specimens captured in October, November, and December (Fig. 1A). Because the number of samples from April consisted of only two skates, we were unable to test for statistical differences between other months. Despite this limitation, the two specimens from April displayed similar values to those in July. Average HSI (Fig. 1B) did not change ($P>0.05$) over the sampling period. However, the average shell gland weight (Fig. 1C) from skates captured in October was greater ($P<0.05$) than those captured in September. Because all shell glands from skates captured in February were in the process of encapsulating ovulated eggs, we were unable to obtain accurate individual shell gland weights.

There were no differences ($P>0.05$) observed in the average diameter of the two largest follicles (Fig. 1D), and no pattern of follicle dynamics was discerned. Also, fully formed egg cases, or those in the process of formation, were found in the uteri of skates captured during all months of the year, except June and September.

Additional analysis revealed that GSI was correlated to shell gland weight ($r=0.53$) and average follicle diameter ($r=0.4$). Furthermore, HSI was also correlated to shell gland weight ($r=0.53$) and average follicle diameter ($r=0.7$).

Assessment of morphological parameters in the male reproductive tract

Histological stages III through VI (SIII–SVI) of spermatogenesis were examined, and GSI and HSI were determined for the 48 males collected during 24 months of sampling. Although the relative proportion of these four stages did not differ among months, it is notable that the production and maintenance of mature spermatocysts (SVI) within the testes persisted throughout the year (Fig. 2A). Similarly, no significant seasonal differences were found in HSI or GSI (Fig. 2, B and C, respectively). In addition, there were weak to no correlations between spermatogenesis and either HSI or GSI (r=–0.07 and 0.13, respectively).

Synchronicity between male and female reproductive cycles

Results from the male and female morphological reproductive parameters indicated that thorny skates are capable of reproducing throughout the year in the western Gulf of Maine. When GSI, follicle diameter in relation to percent composition of SVI, or shell gland weight in relation to percent composition of SVI were compared between male and female thorny skates, no apparent correlation was detected (Fig. 3, A–C). In contrast, when percent composition of SVI (spermatogenesis) was plotted against percentage of captured female skates with egg cases, a strong synchronicity (r=0.51) was observed (Fig. 4).

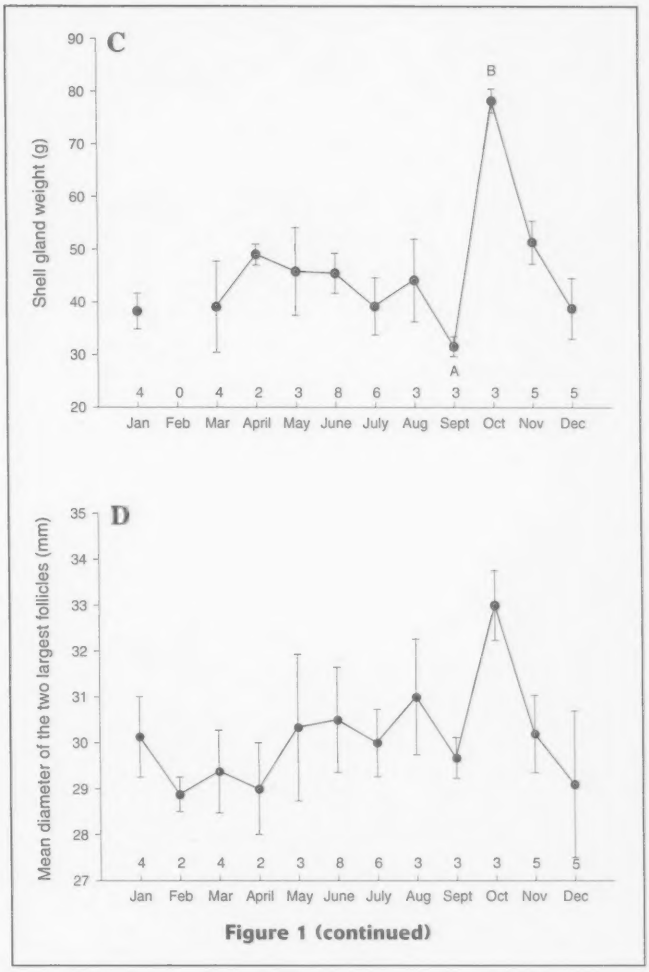

Figure 1 (continued)

Discussion

Elasmobranchs display a wide range of reproductive strategies with morphological and physiological specializations for oviparous or viviparous reproduction (Wourms and Demski, 1993; Hamlett and Koob, 1999). These strategies are associated with one of three basic types of reproductive cycles: 1) reproduction throughout the year, 2) a partially defined annual cycle with one or two peaks, and 3) a well-defined annual or biennial cycle (Wourms, 1977; Hamlett and Koob, 1999). Among oviparous elasmobranchs, some species exhibit cycles with clearly delineated period(s) of reproductive activity interspersed between periods of little or no activity. For example, in the clearnose skate (*Raja eglanteria*), the patterns of estradiol concentrations and follicle dynamics indicate the presence of a well-defined annual reproductive cycle, in which mating and egg deposition take place from December to mid May (Rasmussen et al., 1999). Likewise, hormone and morphological data also indicate a defined annual cycle in the epaulette shark (*Hemiscyllium ocellatum*) (Heupel et al., 1999) and that reproductive activities take place from July to December.

In contrast, other oviparous elasmobranchs exhibit reproductive activity year round. For example, the present study revealed that female thorny skates are capable of reproducing throughout the year. This conclusion was based on GSI, shell gland weight, diameter of the largest preovulatory follicles, and the presence of egg cases in specimens collected over the course of the study. We also observed that GSI and shell gland weight were highest in October. Thus, the period (or periods) of enhanced reproductive activity appears to be an integral part of continuous cycles, although the specific measured parameters or when these periods occur may vary between species.

In a study of thorny skates sampled from August to December in NAFO Division 3N, females were found to be reproductively active over the entire sampling interval, and peak egg case production occurred in September (Del Río, 2002). In contrast, although large preovulatory follicles were present and oviposition occurred throughout the reproductive cycle of the lesser spotted dogfish

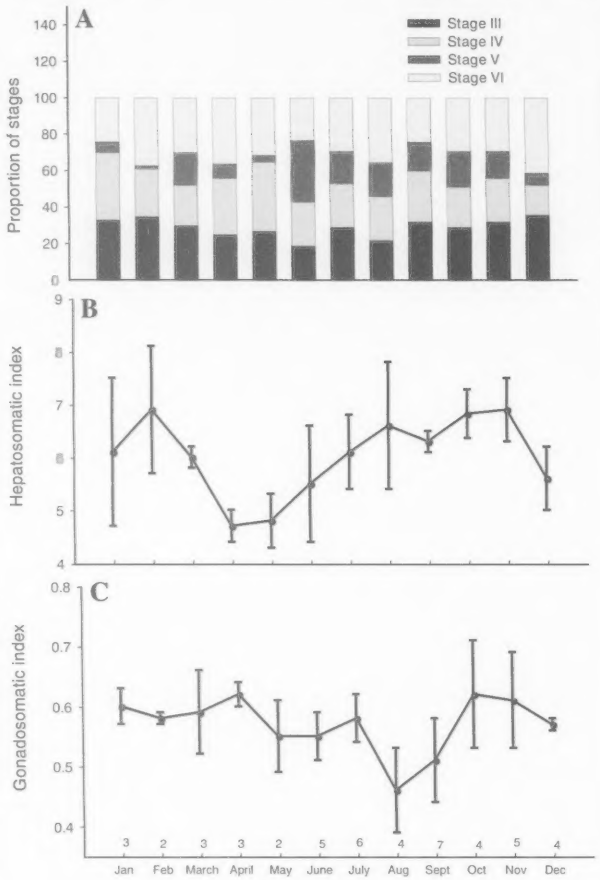

Figure 2

Monthly changes in male thorny skates (*A. radiata*): (**A**) The mean percent of each stage of spermatogenesis (stages III through VI) found along a transect line across one representative full lobe cross section of a testis; (**B**) hepatosomatic index (HSI) and; (**C**) gonadosomatic index (GSI). Sample sizes are indicated above each month. Values are expressed as mean ±SEM.

(*Scyliorhinus canicula*) (Henderson and Casey, 2001), ovary weight and egg deposition peaked during spring. Similarly, several morphological parameters and steroid hormones have been shown to peak in female winter skates (*Leucoraja ocellata*) during the summer, and egg-case production is highest in the fall (Sulikowski et al., 2004). Lastly, in *L. erinacea,* examination of follicle dynamics and egg-case production indicated that a higher proportion of females are reproductively active during two periods of time in the reproductive cycle: in the winter and in the summer (Richards et al., 1963).

The fairly consistent pattern of HSI in female thorny skates over the reproductive cycle indicated that liver reserves (such as lipids and proteins used for oocyte growth) were stored and metabolized continuously throughout the year without a significant change in whole organ biomass. This is in contrast to other oviparous species, such as *S. canicula,* which displayed seasonal variations in liver mass as a result of lipid deposition occurring during different times of the reproductive cycle (Craik, 1978).

The continual presence of mature spermatocysts within the testes over the entire sampling period indicateded that male thorny skates are also capable of reproducing throughout the year. Information describing the annual reproductive cycles of oviparous male elasmobranchs is

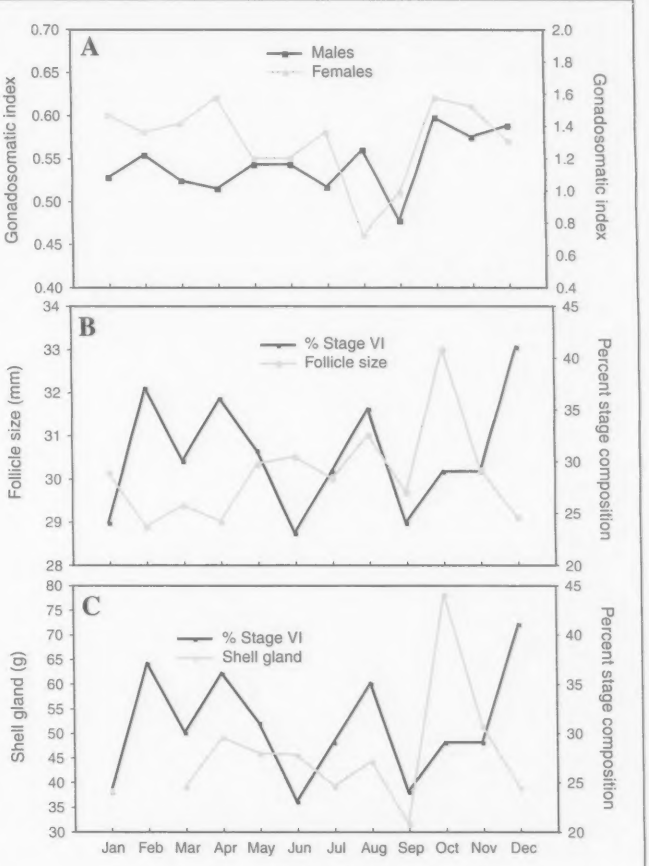

Figure 3

Comparisons between male and female thorny skate (*A. radiata*) reproductive parameters over the course of the sampling period: (**A**) GSI; (**B**) diameter of the two largest follicles and percentage of spermatocysts (SVI) and; (**C**) shell gland weight and percentage of spermatocysts (SVI).

very limited because studies have focused on changes in morphological parameters (i.e., Richards et al., 1963; Craik, 1978) or steroid hormone analyses (i.e., Sumpter and Dodd, 1979; Rasmussen et al., 1999) in females. To our knowledge, the only two species in which quantitative methods were used to describe annual reproductive patterns in males were *H. ocellatum* (Heupel et al., 1999) and *L. ocellata* (Sulikowski et al., 2004). These two species exhibit contrasting strategies in their respective reproductive cycles. For example, similar to male thorny skates from the present study, male winter skates appear capable of continuous production of mature spermatocysts throughout the year (Sulikowski et al., 2004). In contrast, examination of the testes and circulating hormone concentrations in *H. ocellatum* indicated that sperm production and androgen concentration display a concurrent seasonal cycle that peaks from June to October (Heupel et al., 1999).

The lack of correlation between GSI or HSI and stage of spermatogenesis in the thorny skate was not surprising because studies do not support the assumption that relative gonad size (or storage products in the liver) and reproductive readiness are positively correlated (Teshima, 1981; Parsons and Grier, 1992; Maruska et al., 1996). For instance, neither peak sperm production (Maruska et al., 1996) nor the pattern of testosterone concentration was correlated with GSI in *Dasyatis sabina* (Snelson et al. 1997) or *L. ocellata* (Sulikowski et al., 2004).

Relatively few studies have assessed whether cyclical patterns of reproductive morphological parameters or hormone concentrations are coordinated between

males and females over the course of their reproductive cycles. Among them, coordinated peaks in gonad weight and steroid hormone concentrations in winter skates (Sulikowski et al., 2004) and epaulette sharks (Heupel et al., 1999) were observed in males and females over an annual cycle. In the present study, mature spermatocysts (SVI) and percentage of female thorny skates with egg cases were also synchronized over the course of the study. In contrast, Henderson and Casey (2001) found that the gonadal cycles of male and female lesser spotted dogfish were asynchronous, which they hypothesized to be due to the storage of sperm by females. Sperm storage has been documented in other female elasmobranch species as well (e.g., Pratt, 1993; Maruska et al., 1996) and is thought to be a feature primarily of species that are nomadic or segregated by sex (Pratt, 1993). In the current study, *A. radiata* was neither segregated by sex (both genders were captured in the same area and in the same trawls) nor found to be nomadic in their movement patterns (Templeman, 1987; Sulikowski, unpubl. observ.). Moreover, because males are capable of producing viable sperm and females appear to be reproductively active throughout the year, there is probably no need for the population of thorny skates that we sampled to store sperm. On the basis of the above information, we believe that the reproductive cycle in the sampled population of thorny skates is coordinated over an annual cycle.

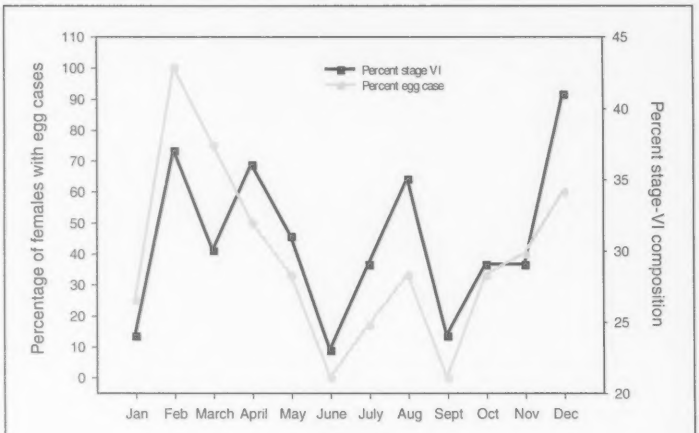

Figure 4

Comparisons between the percentage of spermatocysts (SVI) and the percentage of female thorny skates (*A. radiata*) with egg cases.

In summary, according to the reproductive strategies outlined by Wourms (1977) and later by Hamlett and Koob (1999), the results of the present study indicate that thorny skates have a reproductive cycle that is continuous throughout the year. For females, this conclusion was based on ovary weight, shell gland weight, and diameter of the largest follicles (the preovulatory follicles). For males, this conclusion was based on the presence of mature spermatocysts within the testes over the course of the sampling period. Moreover, comparisons between the proportion of mature spermatocysts within the testes and the percentage of egg-case–bearing females indicate that the reproductive cycles of male and female thorny skates are synchronized. Currently, analyses of circulating steroid hormone concentrations are in progress for the thorny skates used in the present study, which may provide additional insight into the regulation and timing of reproductive events in this species.

Acknowledgments

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Effect of type of otolith and preparation technique on age estimation of larval and juvenile spot (*Leiostomus xanthurus*)

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Otoliths of larval and juvenile fish provide a record of age, size, growth, and development (Campana and Neilson, 1985; Thorrold and Hare, 2002). However, determining the time of first increment formation in otoliths (Campana, 2001) and assessing the accuracy (deviation from real age) and precision (repeatability of increment counts from the same otolith) of increment counts are prerequisites for using otoliths to study the life history of fish (Campana and Moksness, 1991). For most fish species, first increment deposition occurs either at hatching, a day after hatching, or after first feeding and yolksac absorption (Jones, 1986; Thorrold and Hare, 2002). Increment deposition before hatching also occurs (Barkmann and Beck, 1976; Radtke and Dean, 1982). If first increment deposition does not occur at hatching, the standard procedure is to add a predetermined number to increment counts to estimate fish age (Campana and Neilson, 1985).

Accuracy and precision of increment counts is in part determined by the increment formation rate, which has been reviewed elsewhere (Campana and Neilson, 1985; Jones, 1986; Geffen, 1987), and by the type of otolith (asteriscus, sagitta, or lapillus) and the preparation technique used for aging. In most age and growth studies of larval and juvenile fish, the sagitta, the largest of the three otoliths, has been used (Campana and Neilson, 1985), but there are many examples of fish species that can be aged accurately by using the lapillus (e.g., Hoff et al., 1997; Bestgen and Bundy, 1998; Escot and Granado-Lorencio, 1998; Morioka and Machinandiarena, 2001). Although infrequently used, the asteriscus has provided age information with similar or even better precision and accuracy than the sagitta and lapillus (David et al., 1994). However, the microstructure of asterisci is usually not as clear as that of sagittae or lapilli, and the extraction of asterisci is relatively time consuming and laborious (Campana and Neilson, 1985; Neilson and Geen, 1985). As for otolith preparation, two general techniques are common: 1) polishing of one or both sides of a sectioned otolith in transverse view, and 2) polishing of one side of the whole sagitta (Secor et al., 1992). Sagittae and lapilli provide the same accuracy and precision for age estimation; however, lapilli may be easier to process for age determination and may not require processing at all (e.g., Ichimaru and Katsunori, 1995).

Spot (*Leiostomus xanthurus*) is an important fishery species along the southeast coast of the United States (Mercer, 1987) and is a dominant species in coastal ecosystems owing to its abundance (Walter and Austin, 2003). Studies of spot have illuminated processes that affect the abundance of estuarine-dependent species (Warlen and Chester, 1985; Flores-Coto and Warlen, 1993; Ross, 2003). Further, spot has been used as an experimental organism for examining larval ecology (Govoni et al., 1985; Govoni and Hoss, 2001) and otolith chemistry (Bath Martin et al., 2000, 2004; Bath-Martin and Thorrold, 2005). Although spot has been widely studied and is an important ecological and fishery species, basic information necessary for otolith analyses is not available.

Our goal was to provide a foundation for the use of otolith increment counts in examining the ecology of larval and juvenile spot. Our specific objectives were 1) to determine the timing of first-increment formation of spot (*Leiostomus xanthurus*) and 2) to assess the accuracy and precision of age estimates from increment counts made with different combinations otoliths and preparation techniques. Specifically, four combinations of otoliths (sagittae and lapilli) and preparation techniques were compared: 1) a transverse section of the sagitta (polished on one side TSS-1); 2) a transverse section of the sagitta (polished on two sides TSS-2); 3) a whole sagitta (polished on one side WS-1); and 4) a whole lapillus (polished on one side WL-1).

Materials and methods

First increment formation

Six male and six female spot were induced to spawn by injection of human chorionic gonadotropin (HCG) hormone at the NOAA Beaufort Laboratory. Eggs were incubated in a 100-L

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tank at constant temperature (20°C) and salinity (30‰), under 12 h light:12 h dark photoperiod. These conditions were maintained throughout the rearing period. Hatching occurred three days after spawning. Larvae were fed rotifers throughout the experiment and supplemented with enriched *Artemia* from day 20 through day 30. Larvae were collected 4 days (n=5), 12 days (n=7), and 27 days (n=5) after hatching, and live total length (L_T) measurements were made. Larvae were then preserved in 95% ethanol.

Sagittae and lapilli were dissected with fine-tipped forceps and embedded on microscope slides. The increments were clearly visible and otoliths did not require any additional preparation. All increment counts were conducted three times by one person on different occasions with a 100× oil objective and a Nikon E600 microscope with transmitted light. The light was polarized to obtain better visibility. The reader did not know the ages of the fish.

Known fish age and the number of observed increments were used to determine the time of first increment formation on both the sagittae and lapilli. The number of increments deposited between sampling dates divided by elapsed days indicated periodicity of increment formation.

Accuracy and precision

The experimental protocol and conditions were the same as in the previous examination of first increment formation, except that fish were reared for 53 days and artificial diet was added after day 30. Larvae (n=24, 8.8–16.1 mm L_T, mean=11.8 mm L_T) were collected 34 days after hatching, and juveniles (n=34, 19.4–28.1 mm L_T, mean=24.3 mm L_T) were collected 53 days after hatching.

Sagittae and lapilli were dissected from fish with fine-tipped forceps and embedded for sectioning on the transverse plane (right sagitta) or polishing on the sagittal plane (left whole sagitta and lapillus). Priority was given to transverse sections, and if the right sagitta was damaged during preparation, the left sagitta was used (n=8). Otoliths were sectioned with a slow-speed saw with dual diamond wafering blades. Sections were then ground on one side with 1000-grit sandpaper and polished with 0.3-μm alumina paste. After increments were counted on the proximal side of sections that were polished on one side (see below for details), sections were flipped over, ground, and polished to the core to provide a section that was polished on two sides. The left whole sagitta and lapillus were ground and polished in the sagittal plane with 0.3-μm alumina paste. One person made all the increment counts three times for each preparation technique on different occasions with a 100× oil objective on a Nikon E600 microscope with transmitted light. The reader knew the study design, but not the ages of the fish.

The mean number of increments counted from sagittae and lapilli prepared with different techniques were compared with known ages to determine the accuracy of the different aging methods. The statistical significance of differences in increment counts (accuracy) was evaluated with a one-way ANOVA. Increment formation rate was determined by comparing the number of increments counted to known age, and by comparing the difference in the number of increments between 34- and 54-day-old fish and the number of actual days between these increments (20 days).

Precision of increment counts from different otoliths and preparation techniques was determined with the coefficient of variation (CV), calculated by using the three increment counts made for each individual type of otolith and preparation technique (Chang, 1982). The differences in CV values among the four age estimation methods were analyzed by using a Kruskal-Wallis ANOVA. The statistical significance of observed differences were estimated with a *post hoc* Tukey HSD for unequal n test. All the statistical data analyses were performed with Statistica 6.0 software (StatSoft Inc., Tusla, OK).

Results

First-increment formation

First-increment formation on the sagitta occurred at hatching, but there may be problems in resolving increments near the core. Increment counts on sagittae were variable for 4-day-old larvae. Four increments were visible on the sagittae of one individual. The first increment was more pronounced than the others and was interpreted as a hatching check. This increment was approximately 8 μm from the core. On the sagittae of the remaining four 4-day-old larvae, only one increment was visible corresponding to the location of the perceived hatching check. Despite the apparent nondaily increment formation in 4-day-old larvae, an average of 12.3 (range 12–13) increments were visible on the sagittae of 12-day-old larvae, and an average of 26.5 (range 26–27) were visible on the sagittae of 27-day-old larvae. The first increment observed on the sagittae of 12- and 27-day-old larvae corresponded to the location of the first increment observed in the sagittae of 4-day-old larvae (Fig. 1).

First increment formation on the lapillus occurred 6–7 days after hatching. No increments were visible on the lapilli of 4-day-old larvae. In older larvae, an average of 6.4 (range 6–7) increments were observed on 12-day-old larvae and an average of 20.3 (range 20–21) increments were observed on the lapilli of 27-day-old larvae. Additionally, lapilli of 12 and 27-day old larvae exhibited two checks in the area between the otolith core and the first increment, but it was difficult to distinguish which check, if either, was formed at hatching (Fig. 1).

Accuracy and precision

Increments were clearly visible regardless of otolith preparation technique (Fig. 2). Increment width increased from

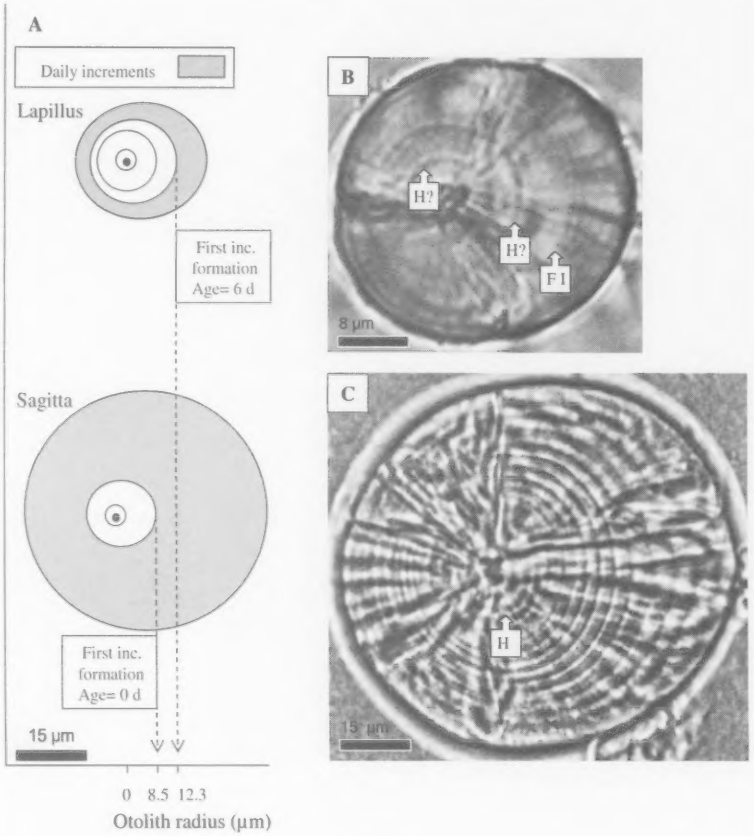

Figure 1

Diagram describing hatching check deposition as well as initiation of daily otolith increment formation in the sagittae and lapilli of spot (*Leiostomus xanthurus*) (**A**). Photographs of the otoliths of a 12-day-old larva: lapilli with six increments (**B**) and sagitta with 12 increments (**C**). Scale bar = 8 µm. H = hatching check; FI = first increment.

the core towards the otolith edge. In both sagittal preparations, increment counts could not easily be made along one radius owing to changes in the growth trajectories (Fig. 2A) and to discontinuities in increment formation (Fig. 2B). However, increment counts could be made along one radius in the lapillus (Fig. 2C)—an advantage that may facilitate measurements of otolith increment widths in future studies.

A hatching check was identified in the sagittae of 34-day-old larvae and 54-day-old juveniles at a location approximately 8.4 µm radius from the core (Table 1). In addition to the hatching check, another well-defined increment was observed in the core area of the sagittal otoliths (Fig. 3A), and this second check was likely related to a dietary switch to exogenous feeding. In most fish the second check was separated from the hatching check by an average of 5.2 increments (n=49, SD=0.59). However, in some fish (n=9), no increments were visible between the hatching check and the other well-defined increment. This observation indicates that there may be problems resolving increments near the core, similar to the results presented above regarding the timing of first increment formation. Owing to the apparent problems discerning increments near the core, the second check was used as a starting point for increment counts. Using the second check as a starting point influenced accuracy but provided a clear starting point for increment counts in all sagittal otolith preparations.

In the lapilli, increment deposition began from a pronounced check visible at ca. 12.3 µm radius from the otolith core (Fig. 3B). This check was found at the same distance from the core in lapilli of 12- and 27-day-old larvae in the experiment on first-increment formation (Table 1). Beginning increment counts from this check would underestimate age by 6–7 days owing to the timing of first-increment formation on the lapilli.

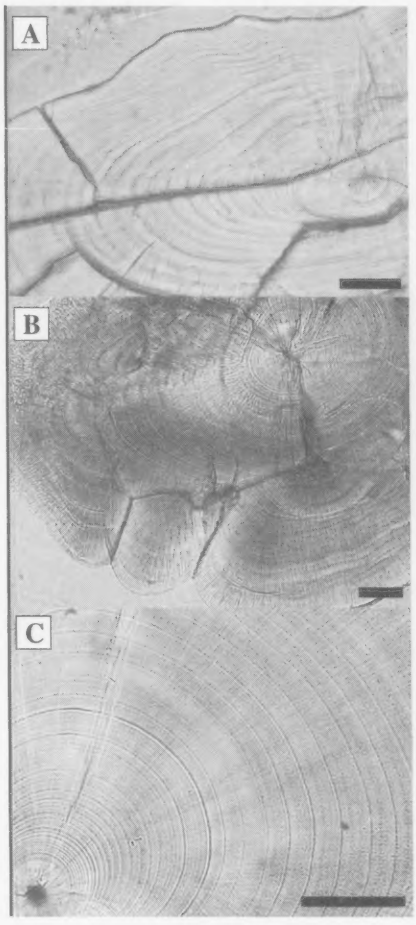

Figure 2

Otolith microstructure of an early-juvenile laboratory-reared spot (*Leiostomus xanthurus*): (**A**) transverse section of the sagitta (polished on two sides); (**B**) whole sagitta (polished on one side); and (**C**) whole lapillus (polished on one side). Scale bar = 30 μm.

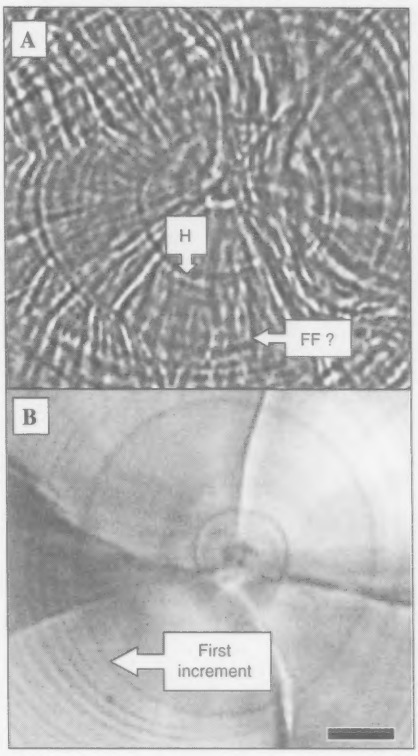

Figure 3

Central otolith area of early-juvenile laboratory-reared spot (*Leiostomus xanthurus*): (**A**) transverse section of sagitta (polished on two sides); five increments are visible between hatching check (H) and, presumably, first feeding check (FF); (**B**) whole lapillus (polished on one side) with daily increments deposited after the check was formed six days after hatching. Scale bar = 10 μm.

Increment formation occurred daily in both sagittae and lapilli after the early larval period. The difference in number of increments counted from sagittae and lapilli from fish sampled 34 and 53 days after hatching reflected the time elapsed between these two samplings (Table 2) and indicated daily increment formation between the larval and early juvenile stage. The same daily increment formation was also observed for larvae sampled 12 and 27 days after hatching during the experiment on first-increment formation (Table 2).

The accuracy of larval age estimates were similar for all the sagittae and lapilli preparation methods (ANOVA, $P>0.05$; Fig. 4A). For juveniles, however, there was a significant difference in the number of counted increments among sagitta preparation methods (ANOVA, $P<0.001$) (Fig. 4B). A lower number of increments were enumerated from transverse sections of sagittae (with one side polished) (*post hoc*: Tukey HSD for unequal n, $P<0.001$). Moreover, ~25% of otoliths within this group were not readable.

All the otolith preparation techniques, except the P1S transverse sections of sagitta from juveniles, underestimated the age from hatching by 9–10 days. A 6–7 day difference was expected between known age and lapilli increment counts, owing to the time of first-increment formation. Thus, actual fish age was underestimated by approximately 2–4 days with lapilli increment counts. A 5-day difference was expected between known age and

Table 1

The distance from otolith core to first increment in the sagitta (first increment formed on the first day after hatching) and in the lapilli (first increment formed six days after hatching) of laboratory-reared spot (*Leiostomus xanthurus*).

		Distance to the first increment (μm)		
Otolith	*n*	Mean	SD	Range
Sagittae—experiment on first-increment formation	17	8.3	0.76	6.7–9.9
Sagittae[1]—experiment on accuracy and precision of aging technique	36	7.8	0.91	6.7–8.8
Lapilli—experiment on first-increment formation	17	12.3	0.54	11.5–13.2
Lapilli—experiment on accuracy and precision aof aging technique	25	12.2	0.61	11.0–14.2

[1] Data for both whole sagittae (polished on one side) along sagittal view, and transverse sections of sagittae polished on two sides.

Table 2

Number of increments deposited on the otoliths of laboratory-reared spot (*Leiostomus xanthurus*) between sampling days in comparison with number of days between sampling days.

Otolith	Sampling days (days after hatching)	Days between sampling	Number of increments between sampling[2]
Sagittae—experiment on first-increment formation	12 and 27	15	14.3
Sagittae[1]—experiment on accuracy and precision of age determination	34 and 53	19	18.3
Lapilli—experiment on first-increment formation	12 and 27	15	14.1
Lapilli—experiment on accuracy and precision of age determination	34 and 53	19	18.6

[1] Data for both whole sagittae (polished on one side) and for transverse sections of sagittae (polished on two sides).
[2] No variance is given because the value represents difference between two average increment numbers obtained for two different groups of fish.

whole-sagittae increments counts, owing to the initiation of increments from a second check, which formed approximately 5 days after hatching. With whole-sagittae increment counts, actual fish age was underestimated by approximately 5 days.

The coefficients of variation (CV), which indicates the precision of age estimates, varied from 1.4% to 8.3% (Fig. 5). CVs were statistically different among age estimation methods for both larvae and juveniles (Kruskal-Wallis ANOVA, $P<0.001$). Lapilli from both larvae and juveniles had lowest CVs, indicating high precision. Whole sagittae and P2S transverse sections for juveniles were comparable, but lower precision for larvae was observed. However, if transverse sections are used for aging, the preparation of both sides is important in the case of larvae (with regard to precision; see Fig. 5) and mandatory in the case of juveniles (with regard to accuracy; see Fig. 4B). In addition, the confidence of the otolith reader in increment recognition (Fig. 5) indicated that the most clear and easy to count increments were found in the lapilli.

Discussion

First-increment formation

In prior studies, the age of larval and juvenile spot was estimated by adding five days to the number of increments counted from sagittae (e.g., Warlen and Chester, 1985; Flores-Coto and Warlen, 1993; Ross, 2003). Our research indicated that increment formation in sagittae occurred at hatching. The only study validating first-increment formation in spot used linear regression analysis for laboratory-reared fish (Peters et al.[1]). The intercept of their regression line (age in relation to number of increments) indicated that the first increment

[1] Peters, D. S, Jr, J. C. DeVane, M. T. Boyd, L. C. Clements, and A. B. Powell. 1978. Preliminary observations on feeding, growth and energy budget of larval spot (*Leiostomus xanthurus*). *In* Ann. Rep. Southeast Fish. Cent., Beaufort Lab. to U.S. Dep. Energy, p. 377–397. Beaufort Laboratory, National Marine Fisheries Service, Beaufort, NC.

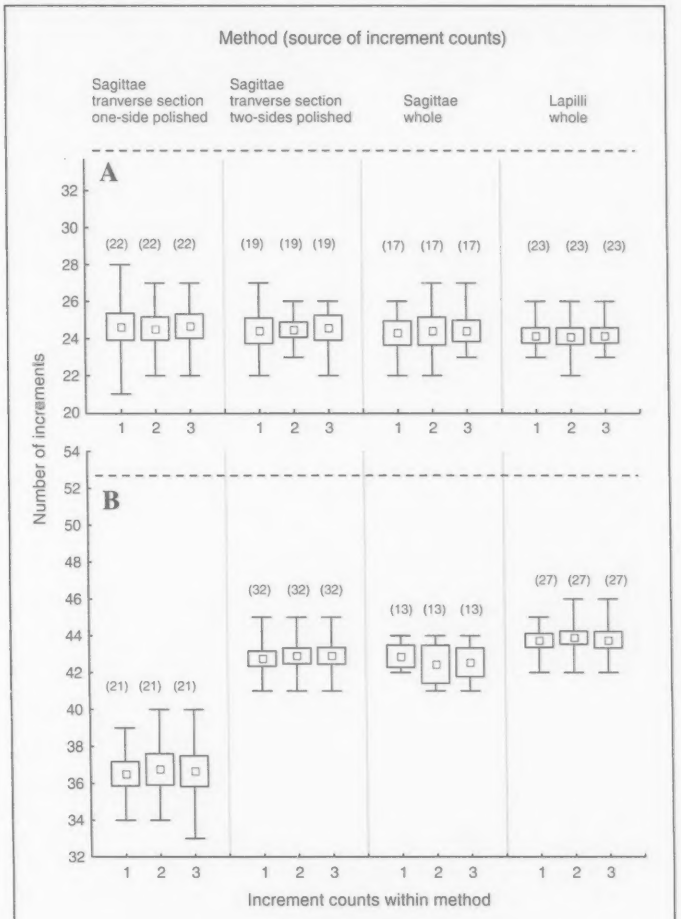

Figure 4

Age of laboratory-reared spot (*Leiostomus xanthurus*) estimated from daily otolith growth increments counted at three different occasions for each preparation method: (**A**) larvae (34 d, 11.8 mm L_T); and (**B**) juveniles (53 d, 24.3 mm L_T). Mean and 95% confidence interval minimum, and maximum values are presented. Values in parentheses indicate sample number. Dashed line indicates the real age.

formed five days after hatching, which corresponds to a time of exogenous feeding initiation in spot (Powell and Gordy, 1980; Powell and Chester, 1985). The other validation experiments on spot (Hettler, 1984; Siegfried and Weinstein, 1989) provided no information on first increment deposition time. In lapilli, increment deposition occurred six days after hatching, but no other studies are available for spot to compare and evaluate these results.

The inconsistency in the time of first increment formation on the sagittae between the present study and Peters et al.'s study[1] may be the result of underestimation by the latter because they did not section or polish the otoliths. Spot otoliths are relatively large and thick and both sagittae and lapilli are difficult to read without otolith preparation for fish older than 25–30 days (~7–9 mm TL). Peters et al.[1] found no increments in sagittae of four- to five-day-old fish. Although in the present study increments were not clear in sagittae of four-day-old spot, fish collected from the same tanks, 8 and 23 days later, had visible increments since hatching. Even if it is difficult to explain why the increments in sagittae of four-day-old-fish were not visible, results presented in the present study support the conclusion that first increment formation occurred at hatching.

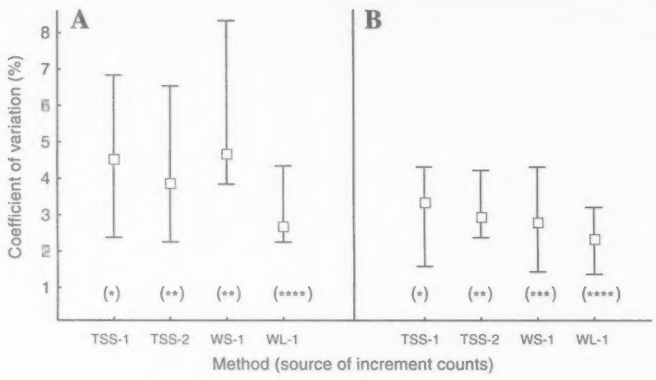

Figure 5

Precision evaluation for different aging methods employed for larval (**A**) and early-juvenile (**B**) laboratory-reared spot: transverse section of sagitta (polished on one side) (TSS-1), transverse section of the sagitta (polished on two sides) (TSS-2), whole sagitta (polished on one side) (WS-1), and a whole lapillus (polished on one side) (WL-1). The coefficients of variation (CV) values were calculated for three independent increment counts per otolith. Additionally, the confidence of the otolith reader in increment recognition has been indicated by numbers of stars, i.e., poor (*), relatively good (**), good (***), and very good (****).

Accuracy and precision of age estimates among different types of otoliths and preparation techniques

Lack of distinct patterns in daily growth increments in otoliths of laboratory-reared fish (e.g., David et al., 1994) could make it difficult to conduct laboratory-based ecological experiments with larval fish. Hettler (1984) attempted to validate increment formation rate in the sagittal otoliths of laboratory-reared spot (13–16 mm SL). Within eight days after tetracycline marking, otolith radii increased approximately 18%, but no increments were observed. Siegfried and Weinstein (1989) confirmed daily increment formation in the sagittae of field-reared spot larvae, but those reared in the laboratory produced 17 increments instead of the expected 30. Our results, on the other hand, provided direct validation of daily increment formation in the sagittae and lapilli of laboratory-reared spot (Table 2).

Even though increment formation was found to occur daily, there were inaccuracies in the estimate of age from otolith increment counts. Twenty-four increments were counted on the sagittae of 34-day-old larvae; if five increments were added for time between first-increment formation and formation of the second check (the starting point of counts used in the present study), age was still underestimated by 4–5 days. Similarly, 24 increments were counted on the sagittae of 34-day-old larvae; if 6–7 days were added to account for the timing of increment formation in the lapillus, age was underestimated by 3–4 days. Similar inaccuracies in age estimates were derived for 53-day-old juveniles. Peters et al.[1] also found age inaccuracies of five days from sagittal increments and concluded that first-increment formation occurred five days after hatching. Given our results and those of Hettler (1984) and Siegfried and Weinstein (1989), we conclude that the likely explanation for age inaccuracies is that the increments near the core of the otolith become harder to read as more otolith material is laid down and this process results in the appearance of fewer increments. These inaccuracies would contribute to a 10–15% underestimation of age from sagittae and a 3–11% underestimation of age from lapilli. To account for these inaccuracies, five increments should be added to increment counts to estimate age.

Lapilli, compared with sagittae, exhibited very clear patterns with increments (Fig. 2) and provided more precise results for the ages of larval and juvenile spot. Although there is no study presenting age data obtained from lapilli for larval or juvenile spot, lapilli have been used successfully for aging many other fish species. Ichimaru and Katsunori (1995) preferred the lapillus as a source of age data for two species of flyingfishes larvae (*Cypselurus heterurus doederleini* and *Cypselurus hiraii*) because increments were as clear as those in the sagittae, yet the lapilli did not require any preparation. Bestgen and Bundy (1998) reported increments deposited on sagittae of Colorado squawfish (*Ptychocheilus lucius*) were difficult to distinguish after fish were 30 days old and thus lapilli were used to age older fish. Lapilli were the preferred otoliths for age determination of young Lost River sucker (*Deltistes luxatus*) and shortnose sucker (*Chasmistes brevirostris*) because of their readability and conservative growth pattern (Hoff et al., 1997). Escot and Grando-Lorencio (1998) concluded

that increments in lapilli of *Barbus sclateri* (Pisces: Cyprinidae) were more clearly defined than in sagittae and asterisci. Similarly, our results demonstrate the utility of lapilli for larval and juvenile fish age estimates.

In addition to the choice of the most suitable type of otolith, the choice of the most appropriate preparation method is an important aspect of larval and juvenile fish age determination (Secor et al., 1992). Analysis of P1S whole sagittae provided in the current study similar precision and confidence in age determination as transverse sections. Although analysis of sagittal transverse sections have been applied to spot (Siegfried and Weinstein, 1989), the most frequently used method has been the analysis of whole sagittae in sagittal view (Hettler, 1984; Warlen and Chester, 1985; Powell et al., 1990; Flores-Coto and Warlen, 1993; Ross, 2003). Recently, Ross (2003) was able to age 40–160 day-old spot juveniles, analyzing whole sagittae along the sagittal view; however, polishing on both sides was frequently necessary. For whole lapilli, however, only one preparation method (i.e., polishing along the sagittal plane) was used in the present study and the results were more satisfactory then those obtained for sagittae and hence no other preparation method (i.e., sectioning) seemed to be required.

In conclusion, first-increment formation occurs at hatching in the sagittae and at 6–7 days after hatching in the lapilli. Increment formation rate occurs daily in both the sagittae and the lapilli. With sagittal and lapillar increment counts, age was underestimated and the cause appeared to be difficulty in discerning increments near the core. Whole lapilli (prepared by polishing one side along the sagittal section) provided age accuracy similar to that of the three sagittal preparations, but higher precision. Future studies would benefit from using the lapillus for ecological studies of the early life history of spot.

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Preliminary use of oxygen stable isotopes and the 1983 El Niño to assess the accuracy of aging black rockfish (*Sebastes melanops*)

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Black rockfish (*Sebastes melanops*) range from California to Alaska and are found in both nearshore and shallow continental shelf waters (Love et al., 2002). Juveniles and subadults inhabit shallow water, moving deeper as they grow. Generally, adults are found at depths shallower than 55 meters and reportedly live up to 50 years. The species is currently managed by using information from an age-structured stock assessment model (Ralston and Dick, 2003).

In many studies, ages are assumed to be accurate and there is no effort to validate the accuracy of the ages (Beamish and McFarlane, 1983). Recent methods of age validation rely upon environmental events that serve as time markers (Campana, 2001). Bomb radiocarbon released during nuclear bomb testing has been used to validate fish ages (Kalish et al., 1996; Campana, 1997; Kalish et al., 1997). Unfortunately, bomb radiocarbon can be used only for fish that lived during the informative period (~1960–70); thus the technique has been used primarily on older ages. For many stock assessments, the validation of younger ages is more critical because of their importance in estimating vital rates, such as growth and maturity schedules.

In this note we apply the well-studied relationship between water temperature and the ratio of oxygen stable isotopes in otoliths to assess the accuracy of young black rockfish ages. Oxygen isotope ratios serve as a record of past water temperatures because the isotope ratio is incorporated into the otolith in near equilibrium with the ratio found in the environment (Patterson et al., 1993; Thorrold et al., 1997) and ambient water temperatures are inversely correlated with $^{18}O/^{16}O$ ratios (Gao et al., 2001). Calcified structures have a strong history of being used in environmental reconstructions based on incorporated trace elements and isotopes (Chivas et al., 1985; Holmden et al., 1997). Otolith microchemistry has been used to successfully reconstruct the environmental history of fish and to answer questions about natal homing (Thorrold et al., 2001) and population mixing (Campana et al., 1999). Variation in oxygen isotopes has been used to confirm visually observed growth increments (Campana, 2001). Recently, stable oxygen isotopes from Pacific halibut (*Hippoglossus stenolepis*) otoliths were used to examine regime shifts in the Northeast Pacific for the identification of changes in bottom water temperatures (Gao and Beamish, 2003). In addition, otolith chemistry may be used to identify environmental events that serve as natural tags for such studies (Campana and Thorrold, 2001). We used a strong regional environmental event, the 1983 El Niño, as a time marker to judge the accuracy of age assignment for black rockfish <15 years of age. The 1983 El Niño produced anomalously warm oceanic conditions along the coastlines in the eastern Pacific; therefore the stable oxygen isotope ratio from 1983 should reflect this change in oceanic conditions.

Materials and methods

We obtained nine pre-aged black rockfish otoliths collected during 1987–91 from recreationally caught fish off Cape Lookout, Oregon (~45.25°N, 145°W), from approximately 15–30 m water depth. One otolith was aged by Oregon Department of Fish and Wildlife scientists by using the traditional break-and-burn method; the matching otolith was used in the stable isotope analysis. Fish from a range of years and ages (Table 1) were selected to include the 1983 El Niño year. A time series of annual summer bottom water temperatures from the same region and depth where the black rockfish otolith samples were obtained, were provided by the Pacific Hindcast from the Columbia University International Research Institute for Climate Prediction, Palisades, New York.

To estimate the year containing the warmest oceanic conditions, we examined otolith material from all opaque growth zones within each otolith for oxygen isotopes ($^{18}O/^{16}O$) and

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Table 1

Age-specific $\delta^{18}O$ values for each black rockfish (*Sebastes melanops*), along with annulus age and collection year. Values replaced by "N/A" indicates samples that were not reported by the stable isotope laboratory.

	Sample number								
Age	1987–33	1987–86	1987–98	1988–7	1988–100	1991–27	1991–86	1991–168	1991–178
0+	−0.96	N/A	−0.633	−0.357	1.209	N/A	−0.128	−2.06	0.756
1+	−1.17	0.914	−0.981	−0.513	0.725	−0.53	−0.114	−1.77	0.948
2+	−0.42	0.654	−0.644	−0.504	−0.544	−0.42	−0.358	−1.09	0.869
3+	−0.04	0.461	−0.518	−0.579	1.037	−0.39	−0.409	−0.64	0.741
4+	0.42	1.006	−0.202	−0.320	N/A	−0.09	−0.082	−0.85	0.338
5+	0.28	0.946	−0.037	−0.257	N/A	−0.22	−0.218	−0.73	N/A
6+		0.957	0.630	0.137	N/A	−0.05	0.1067	0.35	0.785
7+				0.962		1.0	0.512	1.08	1.054
8+						N/A	0.805	1.07	1.113
9+						N/A	1.053	N/A	1.404
10+						N/A	1.164	N/A	1.510
11+						N/A	1.881		
Annulus age (yr)	6	7	7	8	7	12	12	11	11
Collection year	1987	1987	1987	1988	1988	1991	1991	1991	1991

assigned to a year of formation based on estimated age and capture year. Chemical assay and otolith processing were completed at the Stable Isotope Laboratory of the University of Michigan. Each otolith was embedded in epoxy resin and cut transversally with a low-speed diamond-bladed saw. Three or four thin sections ~150 μm thick were removed from the center of each otolith. The thin sections were then glued with cyanoacrilate glue to petrographic glass slides. Samples from multiple thin sections were combined for a single assay. Each opaque growth zone was sampled by using a Merchantek Micromilling system and assays were completed with a Finnigan 251 MAT mass spectrometer. All measurements were reported in standard Vienna Pee Dee Belemnite (VPDB) and notation as δ‰ (per mil), where

$$\delta^{18}O = \left(\left(\left(^{18}O/^{16}O\right)_{sample} / \left(^{18}O/^{16}O\right)_{standard}\right) - 1 \times 1000\right).$$

A time series of $\delta^{18}O$ was constructed for each fish by using the assay from each year-specific sample of the otolith material. Assay results from the collection year were not included because of differences in the season of capture. Years with missing results were due to micromilling or assay errors that resulted in no results reported by the stable isotope laboratory.

To gauge the accuracy of age assignments, we fitted a linear model to each time series and analyzed the residuals from the linear model fit. The $\delta^{18}O$ value corresponding to the negative residual of greatest magnitude from that linear model would be associated with the anomalously warmest oceanic conditions (El Niño). If the age assignments were correct, that portion of the otolith corresponding to 1983 would have the negative residual of greatest magnitude because the observed $\delta^{18}O$ value was much lower than the linear model predicted. Temporal shifts of the most anomalous negative residual with respect to 1983 were interpreted as either an under- or over-estimation of age.

A randomization procedure (20,000 iterations) was used to determine if the magnitude of the average residual in any year was more negative than expected, thus identifying the signal associated with the 1983 El Niño. The residuals from the linear models within each of the fish were randomized with respect to year. Randomized residuals from all iterations were averaged across all fish to produce a distribution of averages. The original year-specific residual averages were compared to the randomization distribution to estimate statistical significance. We rejected the hypothesis of any year with an average residual ≥0 if less than 5% of the randomizations produced a negative average residual of equal or greater magnitude than the observed year-specific average residual, thus identifying anomalously warm years.

An iterative sensitivity analysis was performed by retrospectively removing sequential blocks of years of data and by estimating the statistical significance of the originally determined anomalous years with the reduced data sets. All data taken from years more recent that the cutoff year were removed, and the linear model fitting and randomization procedures were recalculated. The cutoff year was sequentially changed beginning with 1989 to 1986.

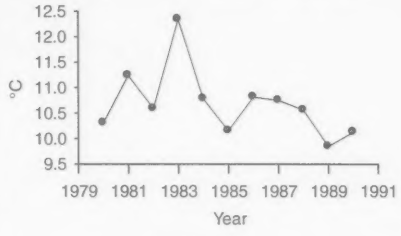

Figure 1

Average summer water temperatures (May–Sep) from Cape Lookout, Oregon.

Table 2

Results of the retrospective analysis that estimated the statistical significance of the magnitude of the average negative residual from the years 1983 and 1985. Assay results from otolith material formed after the cutoff year were removed from the randomization analysis.

Cutoff year	1983	1985
1989	*0.03	*0.05
1988	*0.03	*0.05
1987	*0.04	*0.06
1986	*0.05	*0.26

Results

The period 1980–90 was characterized by an isolated and historically strong 1983 El Niño event (Fig. 1), that resulted in a 1–2°C increase in water temperatures along the Oregon coast. Average summer water temperatures declined slightly over this period. The $\delta^{18}O$ values measured in each fish resulting from this period (Table 1) showed strong patterns that indicated temperature differences both within fish (between years) and between fish (same year). In addition, many fish contained trends in $\delta^{18}O$ across time (Fig. 2). Precision of the reported $\delta^{18}O$ measurements ranged from 0.01 to 0.07‰ (SD).

The residual patterns (Fig. 3A) showed that anomalously warm conditions existed in otolith material corresponding to those of 1983 (n=9, P=0.0338) and 1985 (n=7, P=0.0409). Both old (ages 11–12) and young (ages 6–8) fish appeared to have similar temporal patterns of residuals; however in older fish this pattern shifted by 1–2 years toward more recent years (Fig. 3B). The year-specific averaged residuals of the age 6–8 fish depicted a single anomalously warm year corresponding to 1983. The anomalously warm year in the age 11–12 fish was 1984–85, thus explaining the significance result in 1985. The results of the randomization test were not sensitive to the exclusion of data from the four most recent years (Table 2).

Discussion

The location of the anomalously warm signal in 1983, in the youngest and likely the more accurately aged fish, supports the hypothesis that the 1983 El Niño can be detected by using oxygen stable isotopes. From this analysis, we concluded that the break-and-burn aging method is accurate on average but has a potential tendency toward underaging fish >10 years.

Confirmation of the annual banding pattern in the ololiths of other *Sebastes* species has been accomplished by using a variety of methods. Woodbury (1999) confirmed the accuracy of age assignment in widow (*Sebastes entomelas*) and yellowtail (*S. flavidus*) rockfish, using the change in growth increment width associated with El Niño. Piner et al. (in press) has used bomb radiocarbon to confirm the annual pattern of otolith banding in canary rockfish (*S. pinniger*) and has reported a possible underaging bias for older fish. Andrews et al. (1999) used radiometric age determination to confirm the longevity of long-lived species. However, a larger study on black rockfish with stable isotopes is necessary to conclusively determine age estimates accurately and potential underaging bias.

The 1983 El Niño was chosen for the present study because it was one of the strongest recorded in the century (Sharp and McClain, 1993). Warm water conditions associated with the 1983 El Niño were sufficient to slow growth (MacLellan and Saunders, 1995; Woodbury, 1999) and alter reproductive patterns (VenTresca et al., 1995) in species occupying similar geographic ranges. In contrast, this study attempted to indirectly measure the environment experienced by black rockfish without the need to infer changes to biological processes. Nevertheless, our results appear to support the conclusions of MacLellan and Saunders (1995) and Woodbury (1999) that the anomalous oceanic conditions in 1983 are identifiable.

The analysis of model residuals rather than raw isotope ratios is more appropriate because of the obvious $\delta^{18}O$ temporal trend in some samples. Otolith processing difficulties also may have contributed to the trend. The opaque region of the otolith decreases in size with increasing age. The narrowing of the otolith region associated with older ages made precise sampling more difficult and may have resulted in accidental sampling from otolith material outside the opaque region. The sampling of otolith material from outside the opaque region may have contributed otolith material formed in cooler waters in contrast to the sampling of areas of the otolith associated with younger ages. The increasing trend in $\delta^{18}O$ was not explained solely by the decreasing temporal trend of summer water temperatures. However, an additional component of that trend may be the result of age-dependent fish movement to cooler waters that are deeper or more

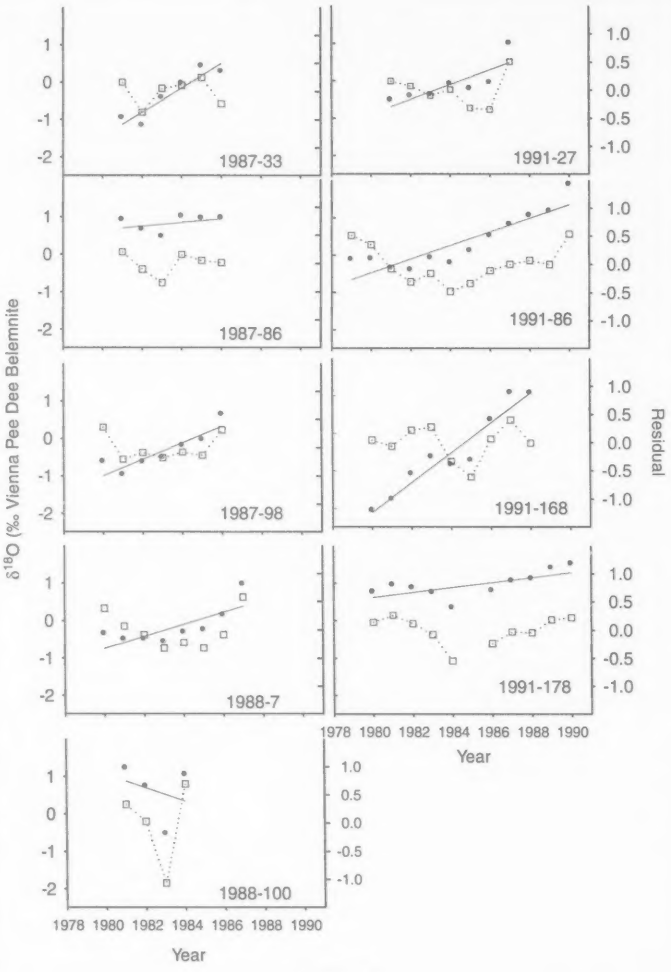

Figure 2

The time series of $\delta^{18}O$ (●, and left axis) and the linear model residuals (□, and right axis) taken from each black rockfish (*Sebastes melanops*) otolith used in the present study. The solid line is the linear model fit to the $\delta^{18}O$ data. A residual value of zero indicates perfect agreement between observed and predicted year-specific average residuals. Sample numbers corresponding to Table 1 are given inside each graph.

northerly. Furthermore, the isotope variability between fish may be due to fish inhabiting different areas in the early periods of life or to temporal differences in growth. Finally, changes in calibration of the spectrometer between assays may be a source of uncertainty.

A critical assumption behind the present study was that the lowest $\delta^{18}O$ corresponds to the warmest water temperature, and consequently the 1983 El Niño that serves as the time marker. The $\delta^{18}O$ values may be impacted by salinity in addition to water temperature (Dorval, 2004), and we assumed that salinity was constant and that the changes in $\delta^{18}O$ values were largely influenced by changes in temperature. A further confounding element to this kind of study is the ability of fish to move and potentially select microhabitats with different temperatures than that of the average local environment. Natural date-specific markers also must be monitored over a number of years to ensure that they remain identifiable within the otolith (Campana, 2001). We addressed this concern by selecting fish of

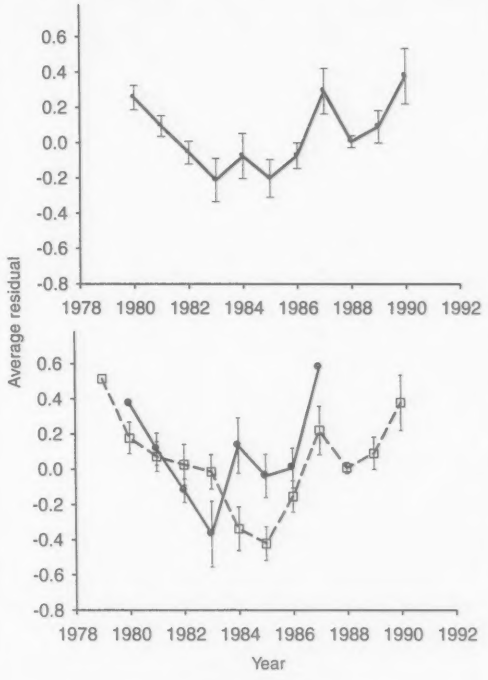

Figure 3

The year-specific average model residual from (**A**) all nine black rockfish (*Sebastes melanops*) and (**B**) ages 6–8 (●, solid line) and ages 11–12 (□, dashed line) fish. Error bars are ±1 SE.

various ages and from various collection dates and by performing the same analysis on each fish. Lastly, this method of age validation can be difficult to implement for fish with small otoliths or for long-lived fish because of the difficulties in obtaining sufficient otolith material from small growth increments.

The detection of El Niño events using $\delta^{18}O$ may allow the use of this reoccurring climatic event as a natural tag for age validation. Because previous studies that used El Niño events as time markers were forced to measure biological reactions to environmental changes, the use of $\delta^{18}O$ may be an improvement because it avoids assuming the intermediate step, namely that environment affects a biological process. The results from this study, however, were based on a small sample size and are, therefore, only preliminary. Further work in this area is warranted.

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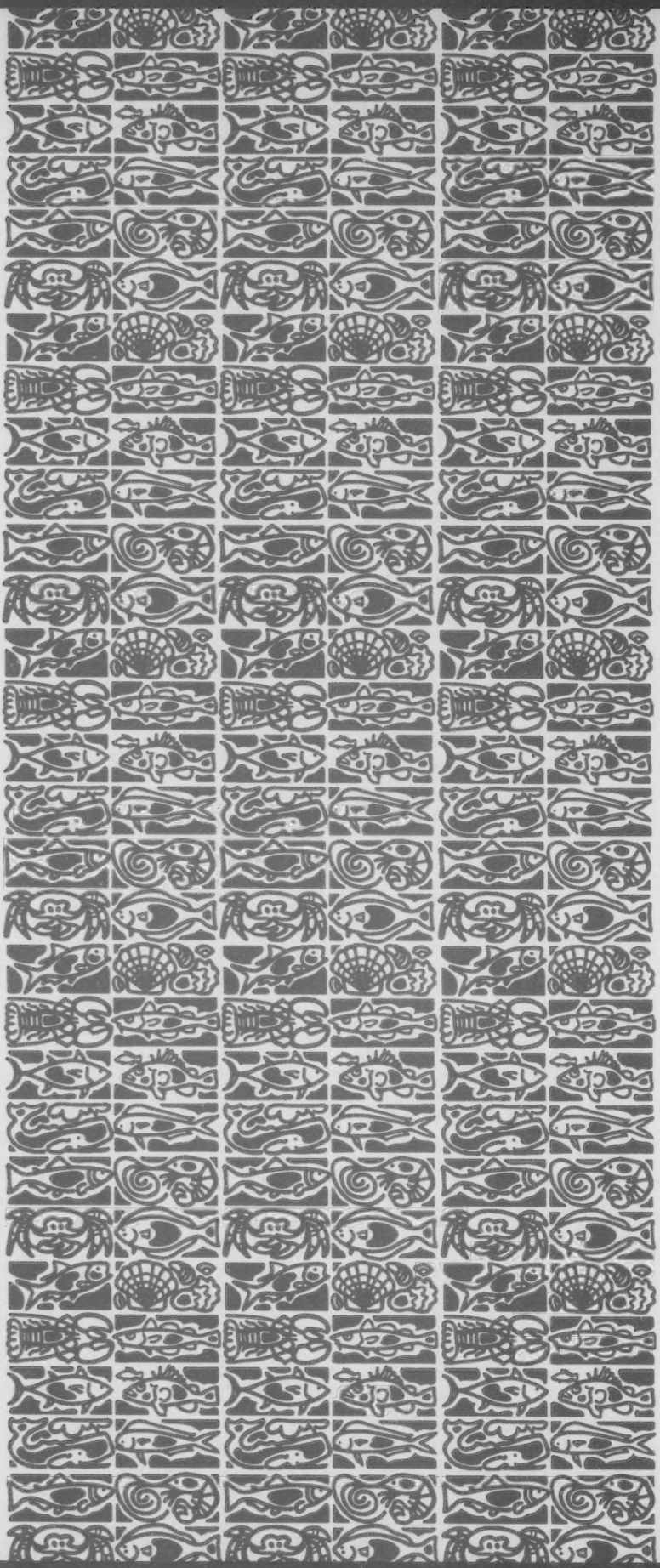
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